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The American Journal of Medicine

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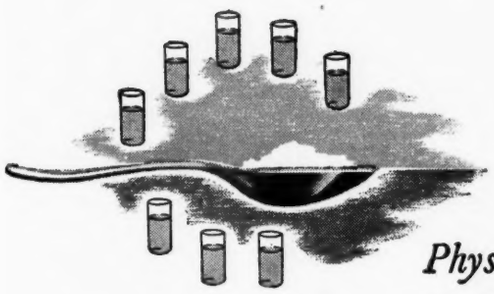

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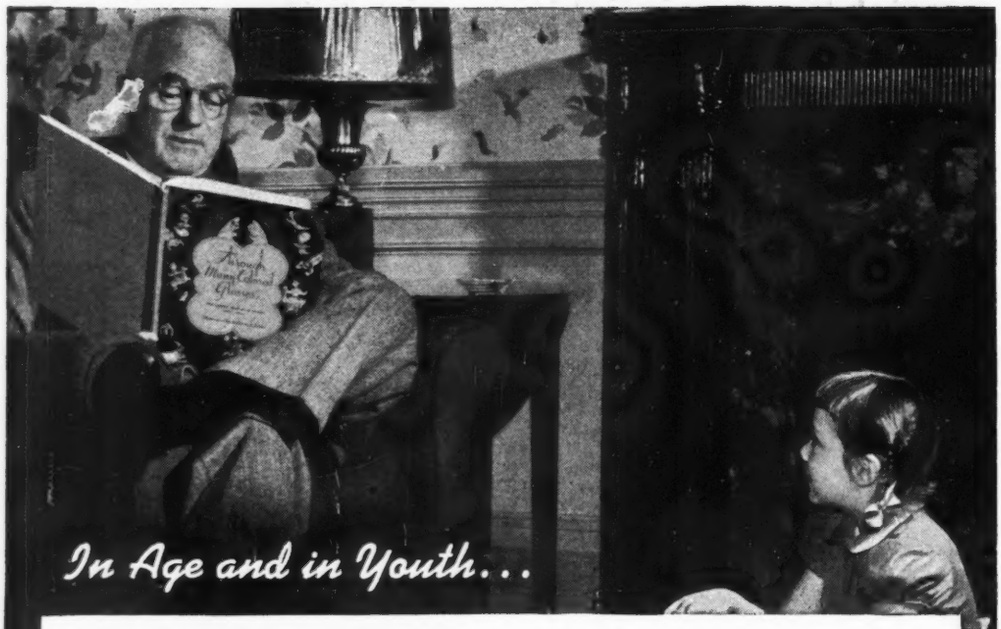

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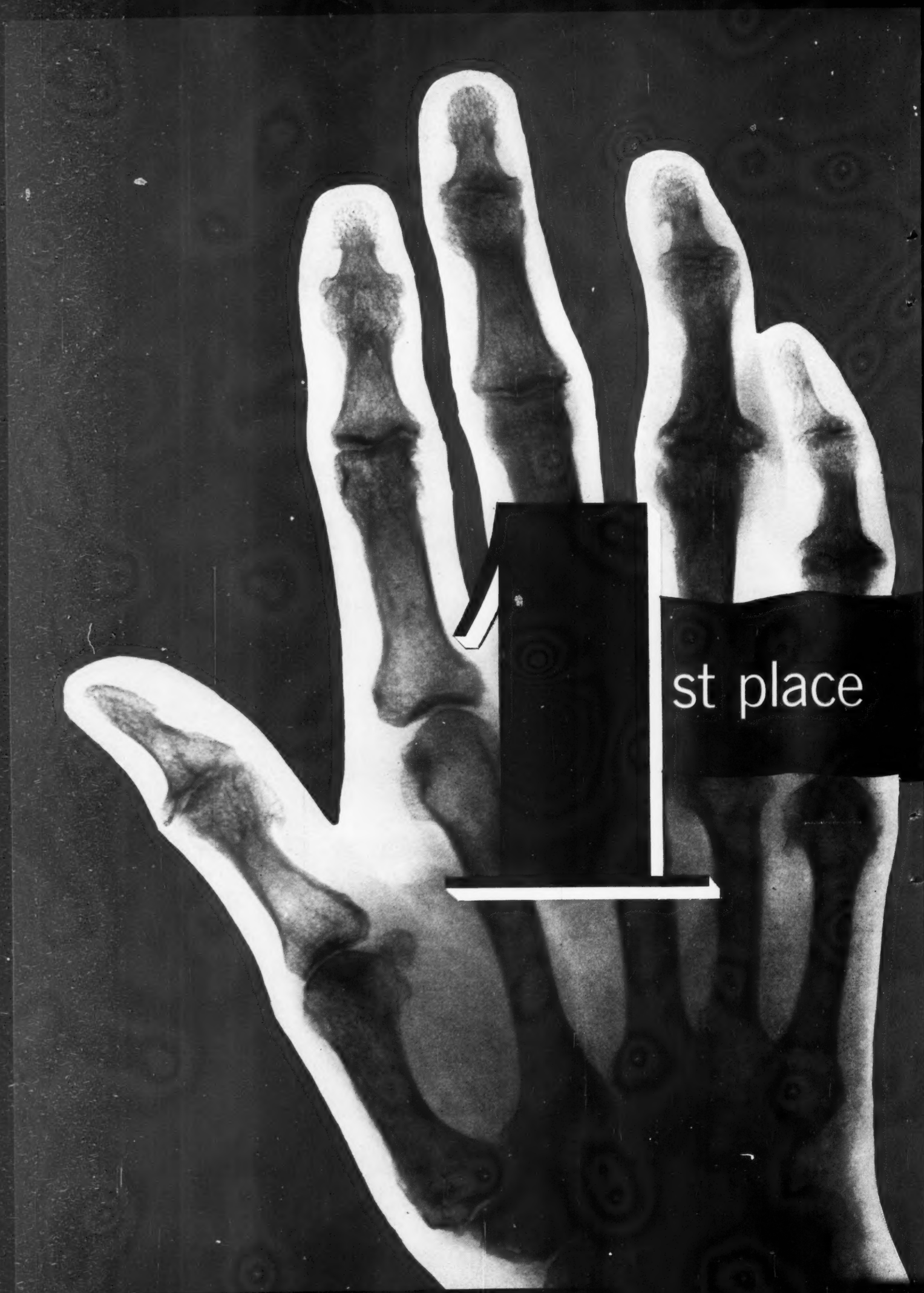
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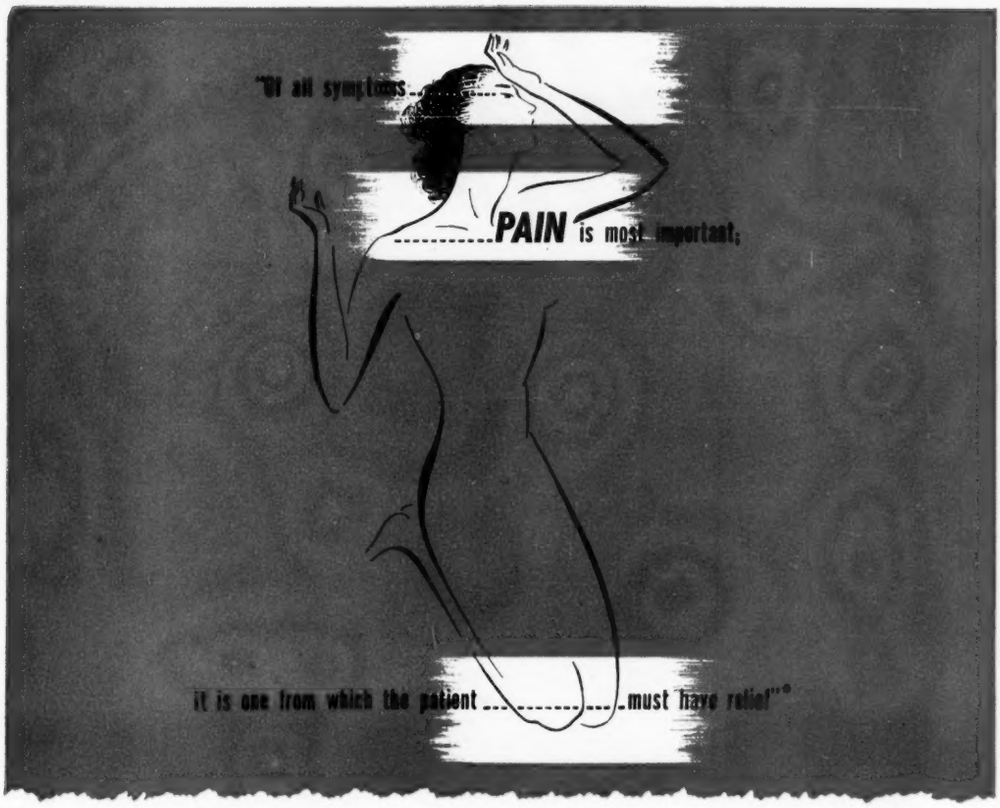

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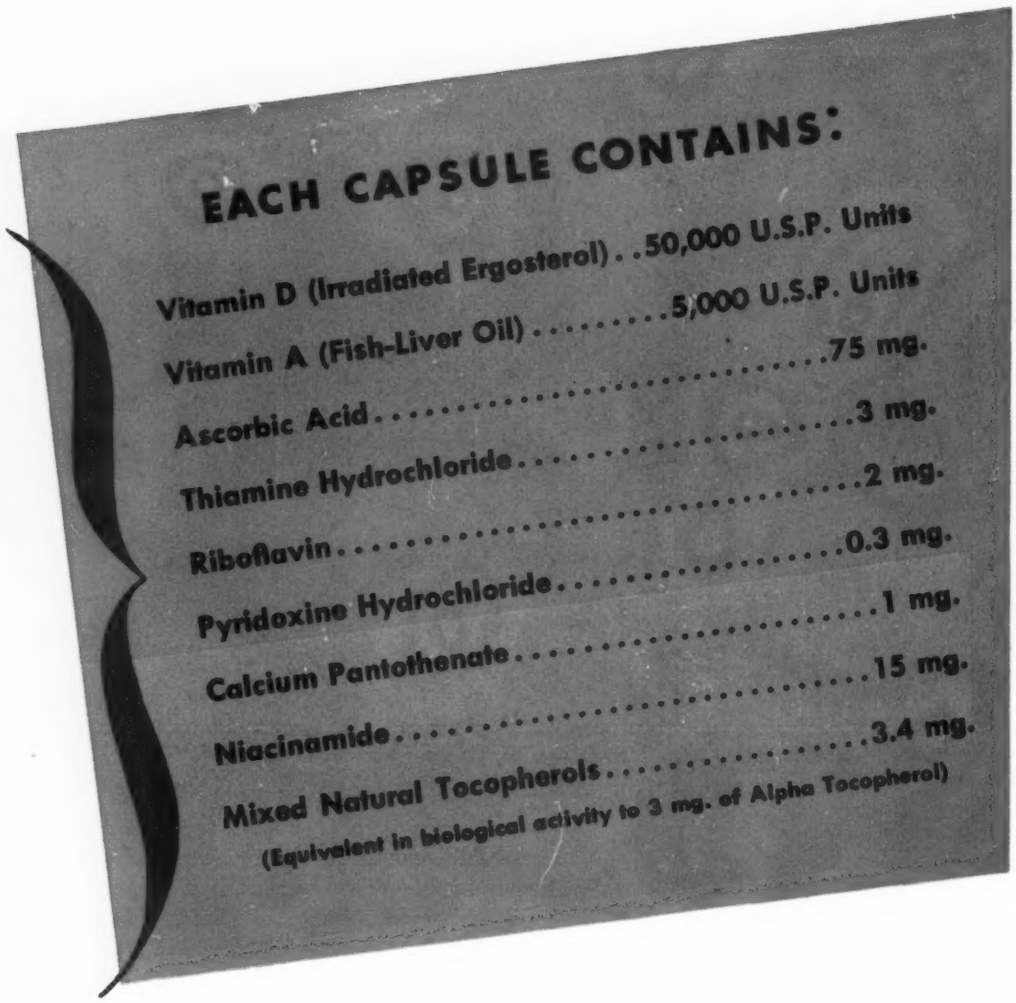

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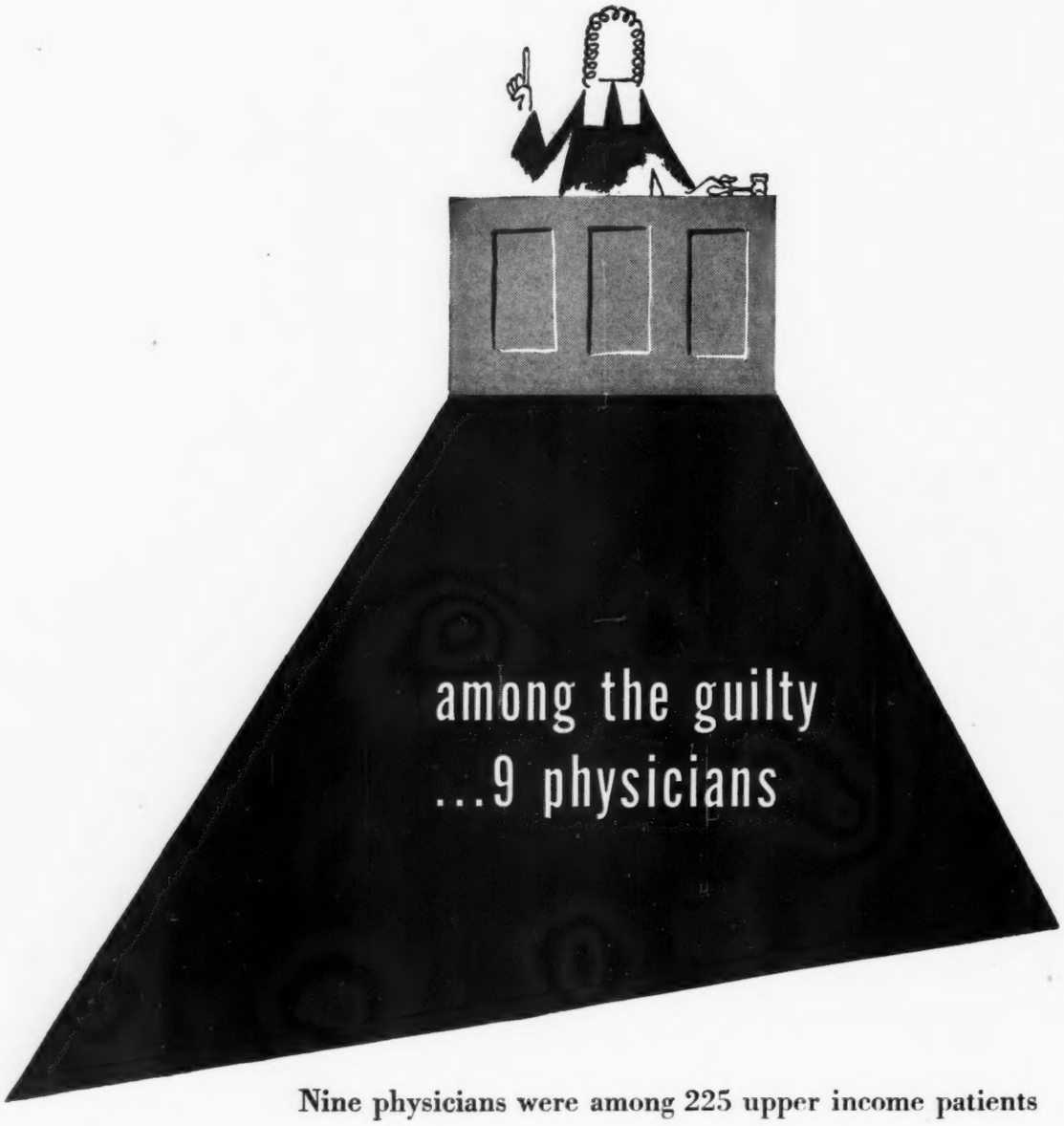

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2. J. A. M. A. **129**:613 (Oct. 27) 1945.

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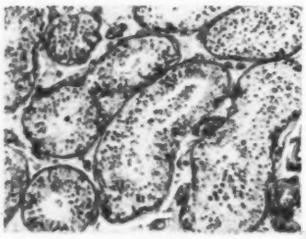
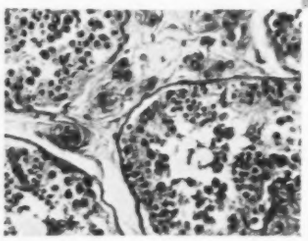
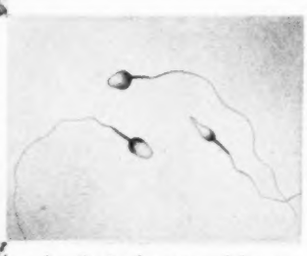
2. Werner, A. A.: J.A.M.A., 132:188, 1946.



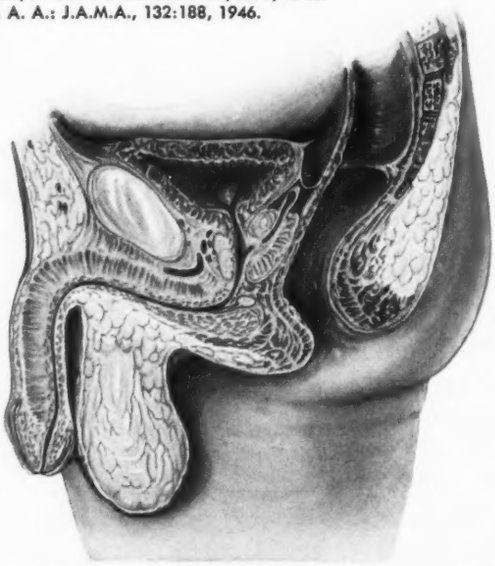
Human testis. Color photomicrograph of transections of tubules showing various stages of spermatogenesis.

Human testis. Photomicrograph of transection of interstitial tissue showing interstitial cells.





Stained smear of human spermatozoa (drawing).



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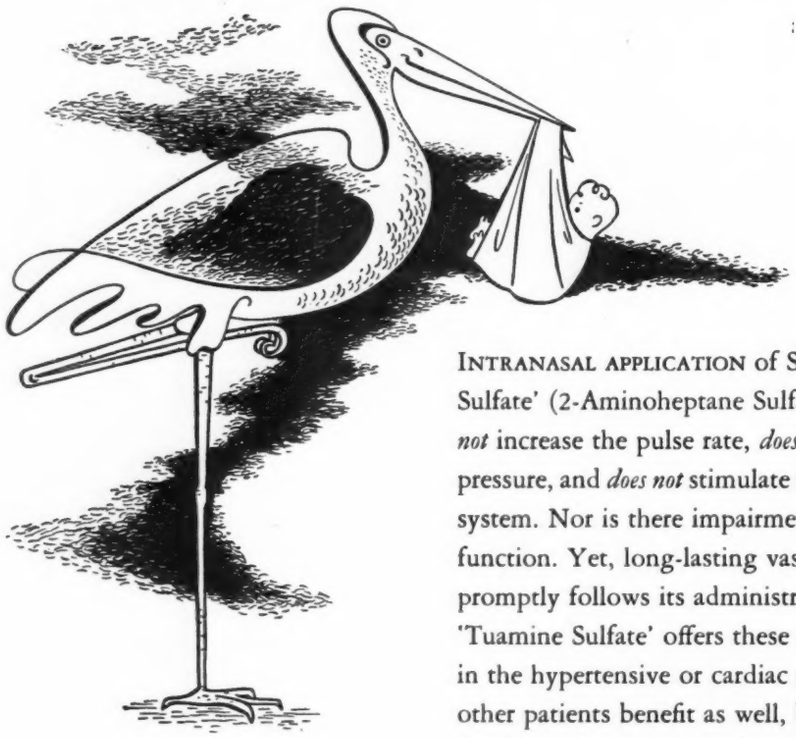

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The American Journal of Medicine

VOL. II FEBRUARY, 1947 No. 2

Potassium Deficiency in a Case of Lymphosarcoma with the Sprue Syndrome*

H. E. HARRISON, M.D., HELEN C. HARRISON, PH.D., R. R. TOMPSETT, M.D.

and D. P. BARR, M.D.

With the technical assistance of Mr. Vincent Toscani

NEW YORK, NEW YORK

THE clinical manifestations of the sprue syndrome have been known since the comprehensive and lucid description by Gee in 1888.[16] The steatorrhea which is its most constant feature is usually evidenced by copious, pale, offensive stools containing large amounts of fatty acids, soaps and neutral fat. The profound emaciation which results from long continuance of the trouble is accompanied by muscular wasting and a great variety of symptoms which may arise from specific deficiencies. Tetany, skeletal deformities, edema, night blindness, hemorrhages, skin eruptions, peripheral neuritis and megaloblastic anemia are abnormalities which appear to depend upon defects in the absorption or utilization of calcium, phosphorus, protein or the vitamins. Together with other manifestations of the condition they present a picture of extensive and serious undernutrition. Little attention has been paid to loss of electrolytes other than calcium and phosphorus in this symptom-complex but the possibility that a loss of potassium may occur was suggested by the finding of a low concentration of serum potassium in two patients.[17]

The sprue syndrome has been described under a variety of names which include celiac disease, tropical sprue, non-tropical sprue and idiopathic steatorrhea. There is still difference in opinion as to whether the celiac affection in children is identical with the disease in adults and whether the syndrome developing in the tropics is of a different nature from that arising in other areas. It seems certain that whenever and however the sprue syndrome may arise, the fundamental defect is one of intestinal absorption, and the clinical manifestations, while quantitatively various, have been qualitatively similar at different ages and in different parts of the world. Almost precisely similar symptoms, moreover, have been observed in two groups of conditions in which pathological cause of the absorptive difficulty may be demonstrated. One group represents a short-circuiting of food from the upper to the lower part of the gastrointestinal tract and has been encountered after operations or as a result of carcinoma of the stomach or colon.[3,6,12] A similar abnormality has been produced in dogs by resection of all or part of the small intestine.[11] The other group has included

* From the Departments of Medicine and Pediatrics, Cornell University Medical College and the New York Hospital.

cases in which there were anatomic lesions of the intestinal lymphatics, of the mesenteric lymph nodes, or of both. In this group are examples of tuberculosis of the lymph nodes,[21,24,28] abdominal Hodgkin's disease, undiagnosed lymphoma with hyperplasia of the lymph nodes, and a case suspected of being lymphosarcoma because of an abdominal tumor and possible lymphosarcoma of the skin.[13] Several other cases having many points in common must be included in this group. The first of these was reported by Whipple in 1907.[34] A medical missionary from Turkey died of a sprue syndrome. At autopsy it was found that the villi of the small intestines were distended by the accumulation of fat-containing multinucleated giant cells and large, foamy endothelial cells. Somewhat similar cases have since been recorded under a variety of titles including malabsorption of fat,[7] mesenteric chyladenectasis[20,26] fat infiltration of the mesenteric lymph nodes[15] and dilatation of lacteals with enlarged mesenteric nodes.[22] The lesions in these cases are so striking that it has been suggested that the sprue syndrome may always be on the basis of lymphatic block,[22] an hypothesis which has little or no anatomic support in the usual case.

This report is concerned with a patient who, during the period of metabolic study, was thought to have uncomplicated nontropical sprue, but who developed thereafter clinical evidence of lymphosarcoma, of which she died.

Two features were of particular interest and prompted the study. First, along with the commonly observed clinical changes associated with sprue, she was found to have a very low serum potassium level. Second, in order to obtain any significant clinical improvement it was found necessary to treat her with massive doses of vitamin D as well as with a low fat, low starch diet.

CASE REPORT

B. K., a Greek housewife, forty-four years of age, was admitted to the New York Hospital on July 21, 1941, with the complaint of diarrhea for nine months and increasing weakness for six months. She was born in Greece. At the age of sixteen she came to this country to be married. She had seven pregnancies in rapid succession. For eighteen months preceding her illness she helped in the kitchen of her husband's restaurant at work of fatiguing nature to which she was unaccustomed. She ate no breakfast, took only a sketchy lunch while she was working and then went home to prepare for the family an evening meal which she was often too tired to eat. She had no known food idiosyncrasies but never drank much milk. In 1924, she had an appendectomy and tubal ligation at Bellevue Hospital. In 1938, she had a contact dermatitis which lasted for almost a year.

She considered herself well until September, 1940, when she gradually developed diarrhea. At first she had only two to four stools a day, but later six or more. The movements were copious, foul-smelling, frothy, usually light in color but occasionally black. No blood or mucus was noted. Occasionally she felt cramp-like pains in the region of the umbilicus. After about three months she became so weak that she was forced to stop work. Her diarrhea continued but was no longer accompanied by cramps. In January, 1941, she consulted a physician who told her that she was anemic. He gave her one injection of a liver preparation, ten intravenous injections of a medicine containing copper and iron, and pills containing iron and vitamins. In April, 1941, he referred her to a hospital where she stayed for two weeks. While there a blood examination revealed 3,100,000 red blood cells, 6,500 white blood cells and a hemoglobin of 60 per cent. An *x*-ray of the abdomen revealed in the right lower quadrant a spherical mass containing large amounts of calcium. This was diagnosed as a fibroid. She was found to have an achlorhydria both before and after histamine and was thought to have pernicious anemia. Her hospitalization brought her no apparent improvement. Her weakness increased. The muscles of mastication

became so fatigued that she could chew her food only with great difficulty. At times she could scarcely lift her arms. Blurring of vision and tinnitus became troublesome. She noted also numbness and tingling in the hands and feet. She received five more injections of liver extract and three transfusions. She was again admitted to the hospital on May 27, 1941, at which time her red blood count was 4,700,000 with 78 per cent hemoglobin. She was given two more blood transfusions and continued on liver injections. On July 9, 1941, her hemoglobin level was 80 per cent, red blood cells 4,800,000.

During this entire period she had no nausea or vomiting and her appetite was excellent. Her diarrhea continued uninfluenced by treatment and her weight fell from 155 pounds in September, 1940, to 105 in July, 1941.

Physical examination revealed a well developed, placid, extremely emaciated woman weighing 43.5 kg. The skin was fine, dry and sallow in color. No abnormal pigmentation was noted. The hair was gray. There was no lymphadenopathy. Examination of the head and neck was negative. The eyes were normal. The tongue was not reddened or atrophic. The lungs were normal. The heart showed no abnormalities except for occasional extra-systoles. Pulse was 86, blood pressure 90/70. The abdomen was moderately distended and the abdominal wall was thin. The liver and spleen were not palpable. There was a firm, slightly movable mass about 5 cm. in diameter in the right lower quadrant, which on pelvic examination was found to move with the uterus but was not definitely arising from it. No other masses were felt. There was generalized muscular wasting, and profound generalized muscle weakness, without spasticity. The Chvostek and Trousseau signs were elicited. The tendon reflexes in the arms and legs were all absent except for knee-jerks, which could be elicited with reinforcement. Superficial abdominal and plantar reflexes were normal. Sensory examination showed no abnormalities.

Laboratory data at the time of admission were as follows: Urine normal. Red blood cells 6,600,000/mm.^{3}, hemoglobin 12.5 Gm., hematocrit 43 per cent, platelets 120,000, white blood cells 9,300 and Kline negative. The stools were bulky, gray, semi-formed, foul-smelling with a negative guaiac test for occult blood. At this time a twenty-four-hour specimen of stool contained 23.5 Gm. of fat, amounting to 30.9 per cent of the dry weight of the stool.

Analyses of the blood serum on admission revealed the following values: total protein, 5 Gm./100 cc.; calcium, 4.8 mg./100 cc.; phosphorus, 2.2 mg./100 cc.; potassium, 1.3 mEq/L; sodium, 139.8 mEq/L. Gastric analysis showed no free HCl in the fasting specimen, and a free HCl of 8 thirty minutes after histamine.

The plasma prothrombin was 63 per cent and plasma vitamin A 58 micrograms per 100 cc. The results of oral and intravenous glucose tolerance tests were as follows:

Blood Sugar Mg./100 cc.

	Fasting	30 min.	1 hr.	2 hr.	3 hr.
Oral	69	92	126	107	94
Intravenous	64	164	111	64	72

Determination of pancreatic enzyme activity in the duodenal contents after intravenous administration of secretin gave the following results, all within normal limits[1]: volume 3 cc./Kg., highest pH 8.15, highest bicarbonate 94 mEq./liter, diastase 10 units/Kg./60 minutes, trypsin 0.87 units/Kg./60 minutes, lipase 162 units/Kg./60 minutes.

Roentgenographic study of the gastrointestinal tract showed a loss of normal mucosal pattern of the jejunum, with apparent spasticity, irregularities of the mucosa and some puddling of the barium. There was a rounded calcified mass in the right side of the pelvis, interpreted as being a calcified fibromyoma.

At the time of admission the patient appeared gravely ill. Her most prominent symptom was extreme weakness. There was no anemia at this time, but her diarrhea continued. She was treated with a high carbohydrate, high protein, low fat diet, parenteral liver extract and added vitamins A, B, C and D, with calcium lactate and potassium citrate by mouth. During the first few months it was necessary to give her calcium intravenously in order to prevent frank tetany. During the first few weeks after admission she

improved a little in that she was a little stronger and able to move about in bed, but there was little change otherwise. Latent tetany was present all the time and the fatty diarrhea persisted. Of particular interest were the changes in the serum potassium which varied from 1.1 mEq/L to 3.2 mEq/L., the usual level being around 2.2 mEq/L. The loss of fat and nitrogen in the stool continued high, the fat varying from 28.8 to 116 Gm. and the nitrogen from 3.8 to 8.3 Gm. per day.

On November 2, 1941, the administration of 500,000 units of vitamin D daily was begun and on January 8, 1942, the dosage of vitamin D was increased to 4,000,000 units a day.* During the next three weeks she showed more striking clinical improvement than at any time previously. Associated with diminution of the diarrhea and increased muscular strength was a return of her serum calcium, phosphorus and potassium to normal or nearly normal levels.

She was placed in the Metabolism Ward in January, 1942, and a series of studies carried out which will be reported in detail below. She remained in the hospital much of the time. While on a low fat, high carbohydrate, low starch diet, with 1,000,000 to 4,000,000 units of vitamin D daily she was at her best, in that her stools were only one or two a day, although still bulky, she was free of tetany, and was strong enough to be up and about most of the day. Her stools were frequently examined for occult blood. Occasionally they were guaiac positive, but almost all were normal. Repeated *x*-ray studies after a barium meal showed the same findings as previously.

In March, 1943, she began to have lower abdominal pains and constipation, and within two weeks developed intestinal obstruction. At operation bilateral pelvic masses were found, and a portion of ileum containing a small mass, thought to be metastatic, was removed. Microscopically, these proved to be lymphosarcoma. She was given *x*-ray therapy over the abdomen, but went rapidly downhill, became deeply jaundiced and died in May, 1943.

Autopsy examination revealed that there was an extensive lymphosarcoma involving the duodenum, jejunum, ileum, colon, liver, right kidney, adrenals, epicardium, myocardium, pericardium, diaphragm, thyroid, uterus, pancreas, pleura and lymph nodes. The lymph nodes chiefly involved were the para-aortic, mesenteric, tracheobronchial, hypogastric and pelvic groups.

There was a large, firm, gray-white tumor mass in the myocardium, also involving the epicardium. The liver weighed 1,850 Gm. and contained numerous tumor nodules. The small intestine showed very striking changes. Beginning at the pyloric ring, the duodenal wall was thickened. There were large masses projecting into the lumen. The ampulla of Vater was invaded. There was slight dilatation of the pancreatic ducts and a few areas of fat necrosis of the pancreas. Throughout the jejunum and ileum there were similar large tumor masses, involving a total of about one-third of the entire wall. Both adrenals contained large masses of tumor, appearing to involve chiefly the medulla. The lymph nodes in the tracheobronchial, pelvic, hypogastric and mesenteric regions were large, firm and gray-white. The lacteals were not dilated. Microscopically all the tumor nodules examined consisted of anaplastic cells with dark nuclei, abundant cytoplasm, many mitotic figures and some tumor giant cells.

METABOLIC STUDIES

These studies were started following the striking improvement in the patient's condition associated with the administration of large doses of vitamin D. At this time the etiology of her steatorrhea was unknown. The extremely low concentrations of potassium in the serum before vitamin D therapy was started were of special interest. Similarly low levels of potassium in the serum had been encountered in another patient with the sprue syndrome[17] and these studies afforded an opportunity to investigate the possibility that the potassium deficiency might be due to excessive loss of potassium in the diarrheal stools.

Methods. All diets were weighed and prepared on the metabolism ward. During

* The vitamin D was given in the form of a concentrated solution of irradiated ergosterol supplied by Dr. C. E. Bills of Mead Johnson and Company.

a given dietary regimen, two diets of approximately the same composition were devised, and these were given on alternate days. Duplicate diets were prepared simultaneously with the diets served to the patient and these duplicate diets were analyzed for nitrogen, potassium, phosphorus and calcium. The fat and carbohydrate of the diet were calculated from standard food tables. Any medication given is indicated in the following discussion, except for vitamin capsules which were administered daily throughout the study. The approximate daily intake provided by this supplement was: Vitamin A 5,000 USP units, thiamin 1 mg., riboflavin 1.2 mg., nicotinic acid 25 mg., pyridoxine 0.116 mg., pantothenic acid 0.48 mg., ascorbic acid 50 mg., and vitamin D 1,000 I.U.

The urine and stools were collected quantitatively, the stools for each period being separated by the administration of carmine red at the end of the period. The periods were of four to six days each. Blood was taken for analysis at the end of each period. The chemical methods used were: potassium in serum, food, urine and stools, Harrison and Darrow;[18] calcium in serum, Kramer and Tisdall;[23] calcium in urine, Shohl and Pedley;[29] calcium in food and stool, Hawk and Bergeim;[19] phosphorus in serum, food, urine and stools, Fiske and Subbarow;[14] nitrogen in food, urine, stools by the macro-Kjeldahl method. Fat was determined in the dried stool by Soxhlet extraction with ether.

The dietary intake during the various periods is tabulated in Table I. The periods during which the massive doses of vitamin D were given are also shown. During periods 1 and 2, the diet was approximately the same as that given for the preceding three weeks, during which time the patient had shown rapid improvement. This diet was high in protein, low in fat and low in starch. No high-starch vegetables were given, and the bread was thoroughly toasted to dextrinize the wheat starch. Most of the carbohydrate intake was in the form of sugars. In the next two periods, 3 and 4, no change was made in the protein and fat intake nor in the quantity of carbohydrate in the diet. The type of carbohydrate fed was changed in that more starch was given in the form of starch-containing vegetables, and untoasted bread. During period 5, the fat content of the diet was increased to 150 Gm. and the protein intake reduced to 75 Gm. per day. The potassium content of the diet was thereby also lowered from 145 mM to 60 mM per day. In period 6 the patient was returned to the diet given in periods 1 and 2. The diet was then kept constant but during periods 7, 8 and 9, the vitamin D supplement of 2,000,000 units daily which had been given heretofore was discontinued. This vitamin D supplement was resumed at the beginning of period 10 and given until the end of the study. In period 16, the diet was modified by reduction of the fat intake from 75 to 50 Gm. The results of the electrolyte and nitrogen balances are shown in Table II.

TABLE I
DAILY DIET AND VITAMIN D INTAKE DURING BALANCE STUDIES

Period	Protein	Fat	Carbohydrate	Potassium mM Per Day	Phosphorus	Calcium	Vitamin D I.U. Per Day
	Gm. Per Day				Mg. Per Day		
1	115	70	350[1]	126	2100	1420	2,000,000
2	115	70	350[1]	126	2100	1420	2,000,000
3	115	70	350[2]	145	2160	1350	2,000,000
4	115	70	350[2]	145	2160	1450	2,000,000
5	75	150	340[2]	60	1850	1410	2,000,000
6	138	75	440[1]	140	2320	1410	2,000,000
7	138	75	440[1]	140	2320	1410	1,000
8	138	75	440[1]	140	2320	1410	1,000
9	138	75	440[1]	140	2320	1410	1,000
10	138	75	440[1]	140	2320	1410	2,000,000
11	138	75	440[1]	140	2320	1410	2,000,000
12	138	75	440[1]	140	2320	1410	2,000,000
13	138	75	440[1]	140	2320	1410	2,000,000
14	138	75	440[1]	140	2320	1410	2,000,000
15	138	75	440[1]	140	2320	1410	2,000,000
16	138	50	440[1]	140	2320	1410	2,000,000

[1] Carbohydrate predominantly in form of sugars.
[2] Proportion of starch increased.

Results. The changes in concentration of potassium, phosphorus and calcium in the blood serum during the course of somewhat over a year of study are shown in Figure 1. During the first few weeks of hospitalization, the patient was given calcium salts by mouth and calcium gluconate intravenously. The serum calcium was raised to almost normal values and the symptoms of tetany ceased. The diarrhea and weakness persisted and symptomatic improvement with increase of muscle strength and lessening of the diarrhea. On the 186th day the first balance period was begun.

The results of the potassium, phosphorus, and calcium balance studies are detailed in Table II and shown graphically in Figures 2, 3 and 4. The height of the column enclosed in the solid line indicates the daily intake of the substance. The vertically

TABLE II

ELECTROLYTE AND NITROGEN BALANCES

Period	Potassium					Phosphorus					Calcium					Nitrogen			
	Intake	Output		Bal.	Serum mM/L	Intake	Output		Bal.	Serum mg./100 cc.	Intake	Output		Bal.	Serum mg./100 cc.	Intake	Output		Bal.
		Stool	Urine				Stool	Urine				Stool	Urine				Stool	Urine	
	mM Per Day					mg. Per Day					mg. Per Day					Gm. Per Day			
1	126	30	78	+18	4.1	2100	933	887	+280	5.7	1420	1440	91	−110	9.2	18.6	3.7	10.0	+4.8
2	126	22	84	+20	4.3	2100	574	1108	+418	5.9	1420	1225	154	+ 41	9.0	18.6	3.0	10.8	+4.8
3	145	57	82	+ 6	...	2160	1230	817	+113	...	1350	1410	97	−160	...	18.1	4.7	10.5	+3.0
4	145	59	87	− 1	3.3	2160	1410	808	− 58	4.7	1350	1500	61	−211	8.3	18.1	5.6	11.1	+1.4
5	60	50	35	−25	2.3	1850	1436	787	−373	4.0	1450	1650	8	−208	7.1	11.6	5.3	8.6	−2.3
6	141	29	68	+44	3.5	2320	1120	799	+401	4.5	1410	1210	16	+184	8.8	22.0	4.3	11.1	+6.5
7	141	51	80	+10	...	2320	1200	990	+130	...	1410	1450	18	− 58	...	22.0	4.7	11.5	+5.8
8	141	69	66	+ 6	3.0	2320	1620	741	− 41	3.6	1410	1745	9	−344	6.1	22.0	6.6	11.0	+4.4
9	141	66	72	+ 3	...	2320	1860	697	−237	...	1410	1550	10	−150	...	25.4	6.1	13.7	+5.7
10	141	51	69	+21	3.0	2320	1333	763	+224	4.3	1410	1425	8	− 23	7.2	23.1	5.3	12.2	+5.7
11	141	43	82	+16	...	2320	1183	1048	+ 89	...	1410	1520	11	−121	...	22.0	5.0	12.6	+4.4
12	141	50	79	+12	3.0	2320	1260	998	+ 62	4.0	1410	1675	10	−275	8.1	22.0	5.3	12.6	+4.1
13	141	51	81	+ 9	3.2	2320	1510	966	−156	4.0	1410	1675	10	−276	8.0	22.0	5.7	13.4	+2.9
14	141	60	70	+11	...	2320	1625	759	− 64	...	1410	1638	11	−239	...	22.0	5.8	12.2	+4.1
15	141	58	68	+15	3.3	2320	1480	801	+ 39	3.8	1410	1610	12	−212	7.3	22.0	5.5	12.4	+4.1
16	141	38	75	+27	3.7	2320	1325	848	+147	4.6	1410	1375	12	+ 23	8.2	22.0	4.7	12.7	+4.6

the serum potassium and phosphorus rose but slightly. When the administration of calcium salts was stopped, the serum calcium dropped. Starting on the 103rd day, 500,000 I. U. of vitamin D were given daily and the dosage was increased to 2,000,000 units daily on the 162nd day, to 4,000,000 units on the 170th day and the dosage decreased again to 2,000,000 units per day on the 182nd day. Following the institution of therapy with massive doses of vitamin D, the concentration of potassium, phosphorus and calcium in the serum rose progressively as shown in the chart. As has been previously mentioned, the patient showed marked

striped portion of the column represents the fecal excretion; the obliquely striped portion, the urinary excretion. The retention is thus indicated by the clear part of the column. A negative balance is shown by the dotted portion of the column extending above the intake line. Each column represents a single period. The spaces between columns represent periods during which the patient was maintained on a regimen identical with that of the following study period but during which no collections were made. Above each column is shown the concentration of the particular electrolyte in the blood serum as determined at the end of the period.

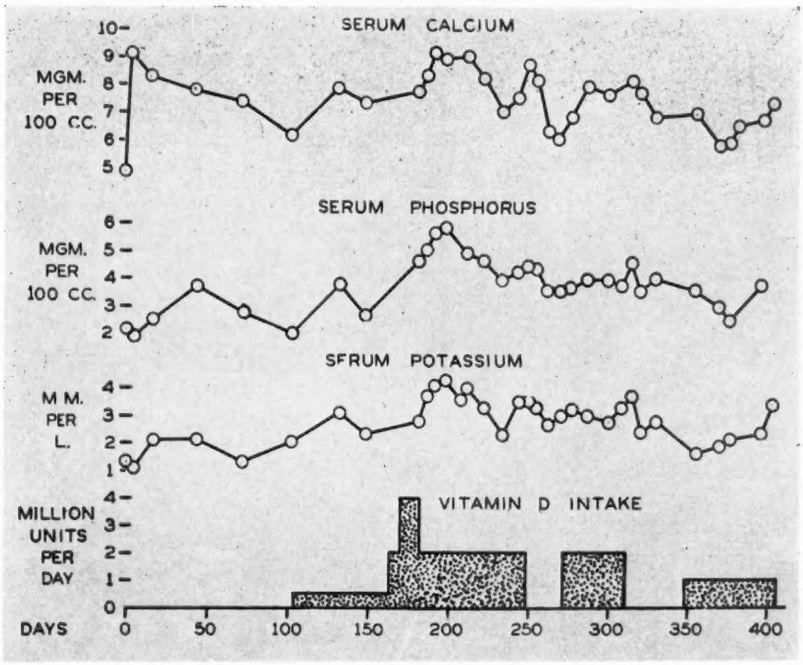

FIG. 1. The influence of vitamin D upon the concentrations of potassium, phosphorus and calcium in the serum.

The changes in the potassium, phosphorus and calcium balances with changes in diet and vitamin D are quite similar. During periods 1 and 2, there was considerable retention of potassium and phosphorus and the patient was essentially in equilibrium with respect to calcium. Following the substitution of starches for sugars in the diet, the diarrhea became more marked and during periods 3 and 4, the fecal loss of potassium, phosphorus and calcium increased. Associated with the increased loss of these substances from the body, the concentration of potassium in the serum decreased from 3.6 to 3.3 mM per liter, that of phosphorus from 5.2 to 4.7 mg. per 100 cc., and the serum calcium dropped from 9.1 to 8.3 mg. per 100 cc. When the fat intake was increased following period 4, the diarrhea became even more marked. The potassium excretion in the stools during period 5 was 50 mM per day, which was almost equal to the potassium intake of 60 mM. The urinary excretion of potassium decreased from an average of 87 mM per day to 35 mM per day. There was still, however, a marked loss of potassium from the body and the serum potassium was reduced to 2.3 mM per liter by the end of period 5. There were also increased losses of phosphorus and calcium from the body during this period, with decreases in the concentrations of phosphorus and calcium in the serum to 4.0 and 7.1 mg. per 100 cc., respectively. During the eight days on this dietary regimen, the patient showed a recurrence of her muscular weakness. When the "optimal" diet was resumed at the end of period 5, there was again a rapid improvement in the patient's status, with a decrease in the diarrhea and increased muscular strength and sense of well-being. On this regimen during period 6, there was a considerable positive balance of potassium, phosphorus and calcium with a rapid increase in the concentration of these ions in the serum.

At the conclusion of period 6, the supplement of vitamin D, which the patient had been receiving for 144 days, was discon-

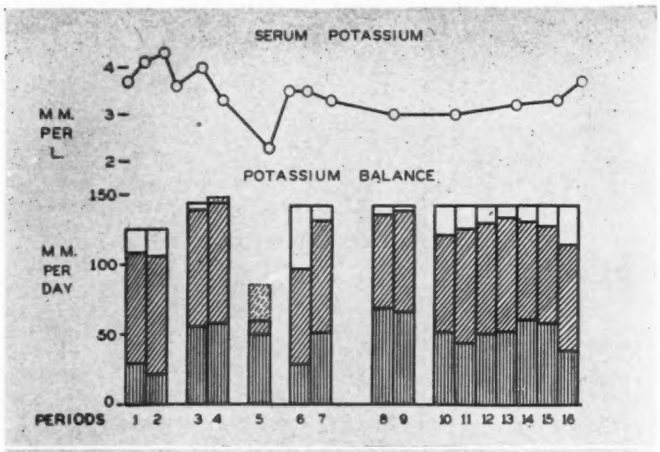

FIG. 2.

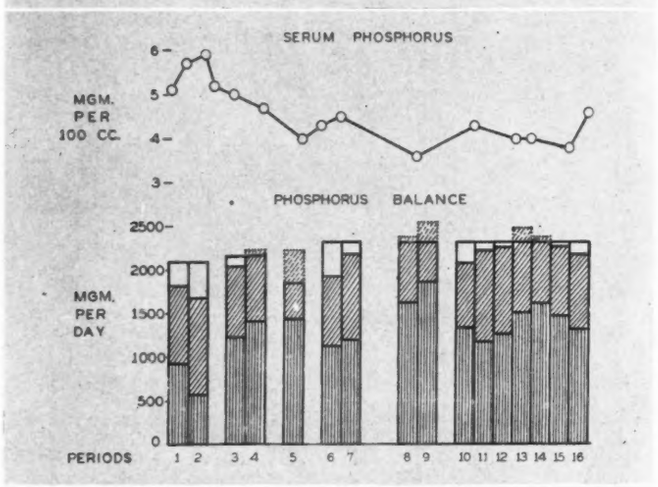

FIG. 3.

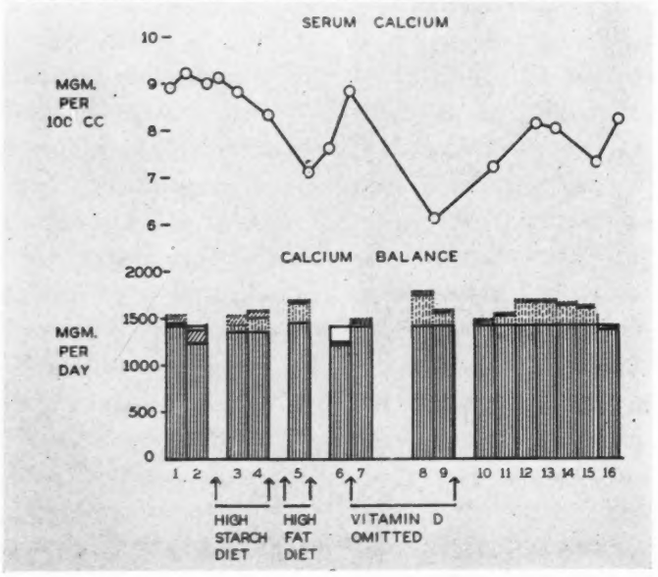

FIG. 4.

FIG. 2. The effect of variations in diet and vitamin D intake upon the potassium balance and the concentration of potassium in the serum. The vertically striped portions of the column represent the fecal excretion, the obliquely striped portion, the urinary excretion. The height of the column enclosed in solid lines indicates the daily intake, and the portions enclosed in dotted lines represent negative balances.

FIG. 3. The effect of variations in diet and vitamin D intake upon the phosphorus balance.

FIG. 4. The effect of variations in diet and vitamin D intake upon the calcium balance.

tinued. No other change was made in her regimen. An almost immediate exacerbation of the patient's diarrhea occurred, with a sharp increase in the fecal excretion of potassium as well as in the loss of phosphorus and calcium. At the end of period 9, vitamin D was again given daily in a dosage of 2,000,000 I.U. per day and definite improvement was noted. The fecal loss of potassium diminished and there was increased retention of potassium. A similar increase in the phosphorus balance occurred. The effect on the calcium balance was less evident. Nevertheless, the serum calcium rose from 6.1 mg. per 100 cc. at the end of period 8, to 8.0 mg. per 100 cc. by the end of period 13. It was evident, however, that the patient's improvement was not being maintained on this regimen as well as it had been at the onset of the metabolic studies. This suggested that the lesion responsible for the disturbance of intestinal function was progressing. Even at this stage, however, reduction of the fat intake to 50 Gm per day in period 16 resulted in increased retention of potassium, phosphorus and calcium associated with improvement in the diarrhea.

The influence of the diet and of vitamin D upon the diarrheal state are further shown by the fat, nitrogen and water content of the stools, which are indicated in Figure 5 as Gm. per day. Even during periods 1 and 2, when the patient was doing relatively well, the stool fat and nitrogen were above the normal values. Following increase of the starch intake in periods 3 and 4, there was a considerable rise in the loss of fat, nitrogen and water in the stools. The absolute loss of fat in the stools was further increased in period 5, when the fat intake was raised from 70 to 150 Gm. per day. Even more striking is the increased loss of water in the stools during this period. The improvement upon return to the low fat, low starch diet is reflected in the decrease

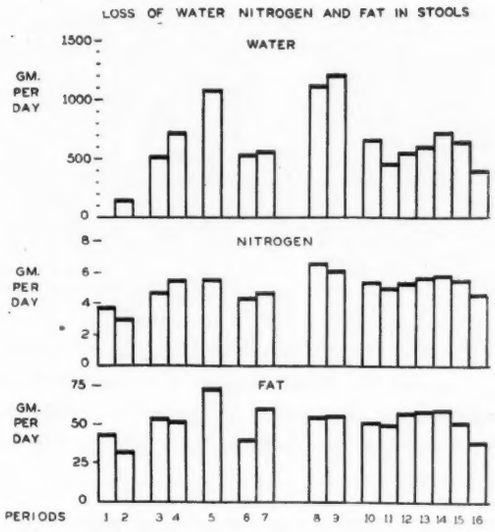

FIG. 5. The losses of fat, nitrogen and water in the stools as influenced by diet and vitamin D intake.

in stool fat, nitrogen and water during period 6. At the end of this period, the administration of vitamin D was discontinued and during periods 7, 8 and 9, the stool fat, nitrogen and water increased with subsequent improvement when vitamin D was resumed at the end of period 9. Despite the continued daily intake of 2,000,000 units of vitamin D daily, the losses of fat, nitrogen and water began to increase again but further improvement was obtained by reduction of the diet fat to 50 Gm. per day in period 16.

The concentrations of sodium and potassium in the stool water are shown graphically in Figure 6. The striped portions of the column represent the concentration of potassium and the clear segment of the column indicates the concentration of sodium. The sum of the concentrations of these two ions ranged from 130 to 177 mM per liter, with most of the values being in the neighborhood of 150 mM per liter. The concentration of the monobasic cations in the stool water is, therefore, approximately equal to the concentrations of monobasic cations in the extracellular water but the concentration of potassium

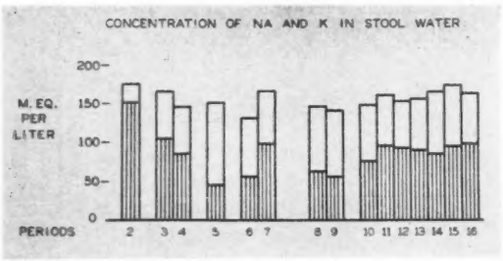

FIG. 6. The concentrations of potassium and sodium in stool water. The striped part of the column indicates the concentration of potassium and the clear portion the concentration of sodium, the total height of the column representing the sum of the concentration of these two ions.

is many times greater in the water of the stools than in the extracellular fluid of the body. The stool potassium was found to be in a diffusible state as indicated by ultrafiltrability through a cellophane membrane.

A deficit of extracellular potassium, as demonstrated by a reduction in the concentration of potassium in the serum, is seen to result from excessive loss of potassium in the stools. It can also be shown that in this patient a deficit of intracellular potassium is associated with decreased concentration of potassium in the extracellular fluid. In Table III the daily potassium and nitrogen balances are averaged for groups of experimental periods during which a given regimen was maintained. In column 1 are given the periods included in each group, in column 2 is shown the average potassium balance, and in column 3 is given the intracellular potassium balance. This is obtained by correcting the total potassium balance for the increase or decrease of extracellular potassium, which is calculated by multiplying the volume of extracellular fluid (0.2 body weight) by the change in concentration of the potassium in the serum during the periods included. No correction was made for change in volume of the extracellular fluid. By comparison with the total potassium balance, the changes in extracellular potassium are small. In column 4 is given the nitrogen balance and in column 5 the theoretical potassium balance calculated on the basis of 3 mM of potassium per Gm. of nitrogen retained or lost. This is approximately the proportion of potassium to nitrogen in intracellular fluid.[9] The "excess potassium" (column 6) is obtained by subtracting the theoretical potassium balance from the intracellular potassium balance. This value indicates the intracellular potassium not accounted for by destruction or repair of body tissue. During period 5, when the concentration of extracellular potassium decreased rapidly, intracellular potassium was lost in excess of the amount expected from the loss of tissue nitrogen. In period 6 the concentration of extracellular potassium was increased and intracellular potassium was retained in considerable amount. During periods 7, 8 and 9, the potassium retention was less than that expected from the nitrogen balance so that a loss of intracellular potassium occurred. When vitamin D was resumed in period 10, the potassium loss ceased and in period 16, there was a considerable increase in intracellular potassium. In general, in those periods in which the concentration of potassium in extracellular fluid dropped, potassium was also lost from the cells and an increase in concentration of extracellular potassium was associated

TABLE III
INTRACELLULAR POTASSIUM EXCHANGE

Periods	Potassium Balance	Intracellular Potassium Balance	Nitrogen Balance	Predicted Potassium Balance	Excess Potassium
	mM Per Day		Gm./Day	mM Per Day	
1,2	+18.6	+18.0	+4.8	+14.4	+ 3.6
3, 4	+ 3.6	+ 4.4	+2.3	+ 6.9	− 2.5
5	−25.4	−23.7	−2.3	− 6.9	−16.8
6	+43.8	+43.8	+6.5	+19.5	+24.3
7–9	+ 7.8	+ 8.2	+5.4	+16.2	− 8.0
10–15	+13.4	+13.4	+4.2	+12.6	+ 0.8
16	+26.6	+25.8	+4.6	+13.8	+12.0

with retention of intracellular potassium. It cannot be determined from our data whether the loss or gain of intracellular potassium was associated with a change in amount of intracellular potassium or whether there was a change in concentration of potassium in the cells. Darrow[9] has reviewed the evidence indicating that potassium may be lost from the muscle cells with replacement by sodium under conditions of potassium deficit and that this process is reversed when potassium is restored.

At the conclusion of period 16 (312th day), the patient was maintained on the diet given during that period but the oral vitamin D was discontinued. Between the 323rd and 330th day, a total of 2,400,000 units of vitamin D was given by intramuscular injection. Despite this treatment, the serum potassium, phosphorus and calcium dropped progressively as shown in Figure 1. On the 348th day, oral vitamin D was renewed in a dosage of 1,000,000 units per day. After a period of about two weeks, the serum potassium, phosphorus and calcium began to increase again. These findings indicated that extremely large doses of vitamin D were necessary to maintain symptomatic improvement and that smaller amounts of vitamin D given parenterally were without effect.

In order to determine whether the vitamin D had been adequately absorbed, the vitamin D content of the blood serum was determined by bio-assay by the method of Warkany[13]. The serum from blood drawn on the 348th day contained approximately 1,000 units of vitamin D per 100 cc. This was at the end of the second period of vitamin D withdrawal and the patient had been given no extra vitamin D for 36 days except that given parenterally.

COMMENTS

In this patient with lymphosarcoma involving the wall of the small intestine and the mesenteric lymph nodes, impairment of intestinal absorption of electrolytes was found to exist along with disturbances of absorption of fat and nitrogen. The defect of calcium and phosphorus absorption associated with fatty diarrhea is well known.[5] Potassium deficiency due to excessive loss of potassium in diarrheal stools has not been recognized as a possible occurrence in steatorrhea. It has long been known, however, that in acute diarrheal disease large quantities of potassium, as well as sodium, may be lost in the watery stools. The concentrations of potassium and sodium in the water of the stools of the patient reported here are similar to those found in acute diarrhea.[10] The disturbances of water and electrolyte absorption parallel to some extent the loss of unabsorbed fat and fatty acids in the feces. When the intake of starch was increased in periods 3 and 4, the loss of fat in the stools increased as did the loss of water, potassium, phosphorus and calcium. This deleterious effect of starch has been found in some cases of fatty diarrhea of unknown etiology classified under the names of celiac disease or idiopathic steatorrhea.[4,25] The increased fat intake and fecal fat during period 5 resulted in an even more marked loss of water in the stools and the loss of potassium in the stools was almost equal to the potassium intake.

The influence of vitamin D upon the intestinal absorption of water and electrolytes was quite striking in this patient. Bassett, Keutmann, Hyde and Van Alstine[5] studied the effect of the administration of 225,000 units of vitamin D daily to several patients with fatty diarrhea, hypocalcemia and hypophosphatemia. They found that vitamin D therapy not only resulted in increased concentrations of calcium and phosphorus in the serum but also in improvement in the diarrhea, with a reduction of the loss of water and nitrogen in the stools. This manifestation was considered by them to be

a non-specific effect of improvement in the patient's state.

In the present study, the bio-assay of the patient's serum thirty-six days after a period of intensive vitamin D therapy indicated that the vitamin D content of the serum was 1,000 units per 100 cc., about ten times the average value in normal adults not receiving extra vitamin D.[32,33] In arthritics given up to 500,000 units of vitamin D daily, concentrations of vitamin D in the serum as high as 9,000 to 13,000 units per 100 cc. have been found, with concentrations of 2,000 to 4,000 units one month after therapy was stopped.[33] It is likely that the vitamin D given to this patient was not completely absorbed. Lack of absorption alone, however, cannot explain the need for such large amounts of vitamin D. When the administration of vitamin D was discontinued the first time, the serum calcium and phosphorus dropped rapidly and the diarrhea became more marked within a few days. During the second period of lack of vitamin D, the serum calcium and phosphorus dropped despite the intramuscular injection of 2,400,000 units of vitamin D. The serum calcium was 6.9 mg. per 100 cc. at a time when the blood serum contained ten times the average normal concentration of vitamin D. The findings suggest that extremely high concentrations of vitamin D in the serum were necessary for the effect of vitamin D upon the intestinal function seen in this patient.

It is difficult to say to what extent the potassium deficiency shown by this patient contributed to her symptoms. Both she and a previous patient with potassium deficiency associated with the sprue syndrome[17] exhibited marked muscle weakness, hypoactive deep reflexes and hypotension. In view of the other nutritional disturbances present due to the chronic diarrhea, one can only speculate about the possible relationship of potassium deficiency to the impairment of muscle strength. In dogs, severe muscle weakness and paralysis may result from a diet low in potassium.[27] In man, the syndrome of familial periodic paralysis is known to be associated with reduction of the serum potassium.[2,30] Extremely low concentrations of serum potassium have been found, however, by Butler, Talbot and MacLachlan[8] in children given testosterone propionate without any symptoms which could be ascribed to the low concentrations of extracellular potassium. The decrease in extracellular potassium in patients receiving testosterone propionate was thought to be due to rapid synthesis of cell protein with retention of intracellular potassium. In the potassium deficiency resulting from loss of potassium in the diarrheal stools, the evidence indicates that loss of intracellular potassium is associated with deficiency of extracellular potassium. If potassium salts had been administered to these patients without other therapy, it might have been possible to ascertain whether any therapeutic effect was produced by replacement of the potassium deficit alone. Unfortunately, this was not done.

The disturbance of intestinal function in this patient was presumably the result of injury to intestinal mucosa by infiltration with lymphosarcoma and obstruction of the lymphatics. Obstruction of the pancreatic ducts was not present during the period of study as indicated by the normal content of pancreatic enzymes in aspirated duodenal contents.

SUMMARY

A patient with chronic diarrhea was studied who showed marked reduction of the concentrations of potassium, calcium and phosphorus in the serum, associated with excessive losses of fat, nitrogen, water, potassium, calcium and phosphorus in the stools. At autopsy, a lymphosarcoma of the small intestine and mesenteric lymph nodes

was found which was the basis for the disturbance of intestinal function.

Balance studies were made on various dietary regimens. These studies indicated that the loss of potassium in the stools was responsible for the reduction of serum potassium found in this patient. Comparison of the nitrogen and potassium balances indicated that during periods of potassium deficit, intracellular potassium was lost in excess of that expected from nitrogen loss.

The severity of the diarrhea was reduced by a diet low in fat and in starch. Large doses of vitamin D were found to be effective in further reducing the loss of water, potassium, calcium and phosphorus in the stools.

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A Clinical Comparison of the Effectiveness of 6-n-Propylthiouracil and 2-Thiouracil as Antithyrotoxic Agents*

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MANY compounds have now been tested for their goitrogenic influence upon small laboratory animals.[1,2,3,4,5,6,7,8] The activity of any particular substance seems to depend upon a —N—C—X group, where X is an N, O or S atom. The most effective agents are those in which a complete thiourylene radical is present. Astwood found 115 of 220 compounds goitrogenically potent and twenty-five of these "as active or more active than thiouracil" weight for weight.[1] Of these twenty-five, 6-n-propylthiouracil (hereafter designated propacil) was the most powerful and eleven times as goitrogenic in the rat as 2-thiouracil (hereafter designated thiouracil).[1]

Inasmuch as results in the animal cannot be depended upon to hold for the human being, extensive clinical trial and comparison must be carried out for each individual agent which appears promising for its antithyrotoxic effect. For instance, Astwood's report[9] indicates that propacil does not compare as favorably with thiouracil in man as it does in the rat, being only five times as active as the latter in the control of toxic goiters. In view of the all too frequently occurring severe reactions [10,11,12,13] to thiouracil, any less toxic agent such as propacil has been shown to be in preliminary clinical trial[9,14,15] will be especially welcome for the management of thyrotoxic subjects. The present analysis of cases in which the patients were treated with propacil may encourage its more widespread application in the management of hyperthyroidism.

MATERIALS AND METHODS

Eighteen male and fifty-seven female subjects with thyrotoxicosis were studied while under treatment with propacil (Table 1) for periods ranging from two to twelve months. Thirty-three of these were hospitalized for varying periods in order to allow more thorough investigation but without relation

TABLE 1
AGE AND SEX DISTRIBUTION IN SEVENTY-FIVE CASES OF THYROTOXICOSIS TREATED WITH 6-N-PROPYLTHIOURACIL

Type of Goiter	Total	Male			Female		
	No.	No.	Age (Yr.)		No.	Age (Yr.)	
			Range	Av.		Range	Av.
Hyperplastic..	41	8	27–52	41.3	33	18–47	39.8
Nodular.....	34	10	29–51	53.4	24	23–75	48.2

* From the New York Medical College, Metropolitan Hospital Research Unit, Welfare Island, New York.

necessarily to the degree of toxicity exhibited by them. Propacil was administered in 25 mg. tablets* from one to several times daily.

The criteria necessary to establish a diagnosis of toxic goiter and other features of the regimen, including a description of the laboratory methods used, have been previously described.[11,16]

RESULTS

In all, propylthiouracil has been administered to more than 100 patients. However, guided by the experience of others,[9] we used too little of the drug at first so that some of these patients were operated upon or returned to the use of thiouracil.

CLINICAL DATA

In the seventy-five patients whose symptoms of toxic goiter were brought fully under control by the drug, the symptoms and qualitative therapeutic responses were similar in every regard to those obtained with thiouracil. These were detailed at some length for the latter drug in a previous study.[11] Therefore, only the more essential data will be included here.

The women ranged in age from eighteen to seventy-five years, the men from twenty-seven to fifty-two (Table I), with averages of 40.7 and 46.9 years, respectively. Fifty-one had a predominantly hyperplastic gland; thirty-four had a nodular type of goiter. The response of the basal metabolic rate in relation to dosage is shown graphically in Figure 1 and closely follows the "iodine decay curve" of Means and Lerman.[17] The average initial or pretreatment basal metabolic rate in forty-two previously uncontrolled patients was plus 49.0 per cent; when fully controlled, the average rate was plus 8.6 per cent.

* Generous supplies of 6-n-propylthiouracil were courteously supplied by Dr. Stanton Hardy of the Lederle Laboratories. Recently this material has been available as a 50 mg. scored tablet, so that doses of 25 mg. may be easily administered.

DOSAGE

In regard to dosage, our patients may be divided into two groups: (1) forty-two who had received no previous chemotherapy and (2) thirty-three who had been previously rendered non-toxic by the administration of thiouracil.

In the first group, effective initial daily doses ranged from 75 to 250 mg. with an average of 165 mg. (Fig. 1.) For both groups the maintenance dose varied from 25 to 75 mg., with an average of 55 mg. per day.

The time necessary to bring about complete control in the patients who had not previously received thiouracil varied from two to seven weeks, being generally, although not invariably, longer with the smaller initial doses. However, each of the ten patients who received 200 mg. or more daily at the beginning was controlled in four weeks or less.

The doses necessary to maintain a normal production of thyroid hormone in the thirty-three patients previously controlled by thiouracil varied from 25 to 100 mg. daily. The actual amount given was perhaps less informative than the ratio of that amount to the dose of thiouracil previously required. That ratio varied from 1:1 to 1:4. (Table II.) In general it would appear that ratios of from 1:2 to 1:3 were most effective. In other words, weight for weight, propacil is from two to three times as effective as thiouracil.

TABLE II
WEIGHT RATIO OF EFFECTIVE DOSES OF 6-N-PROPYLTHIOURACIL AND 2-THIOURACIL IN THIRTY-THREE PATIENTS

Propacil: Thiouracil Ratio	1:1	3:4	1:2	1:2.5	1:3	1:4
No. Cases	1	1	12	6	8	5

From our experience with more than 100 patients treated with propacil and over 200 treated with thiouracil, we have found the

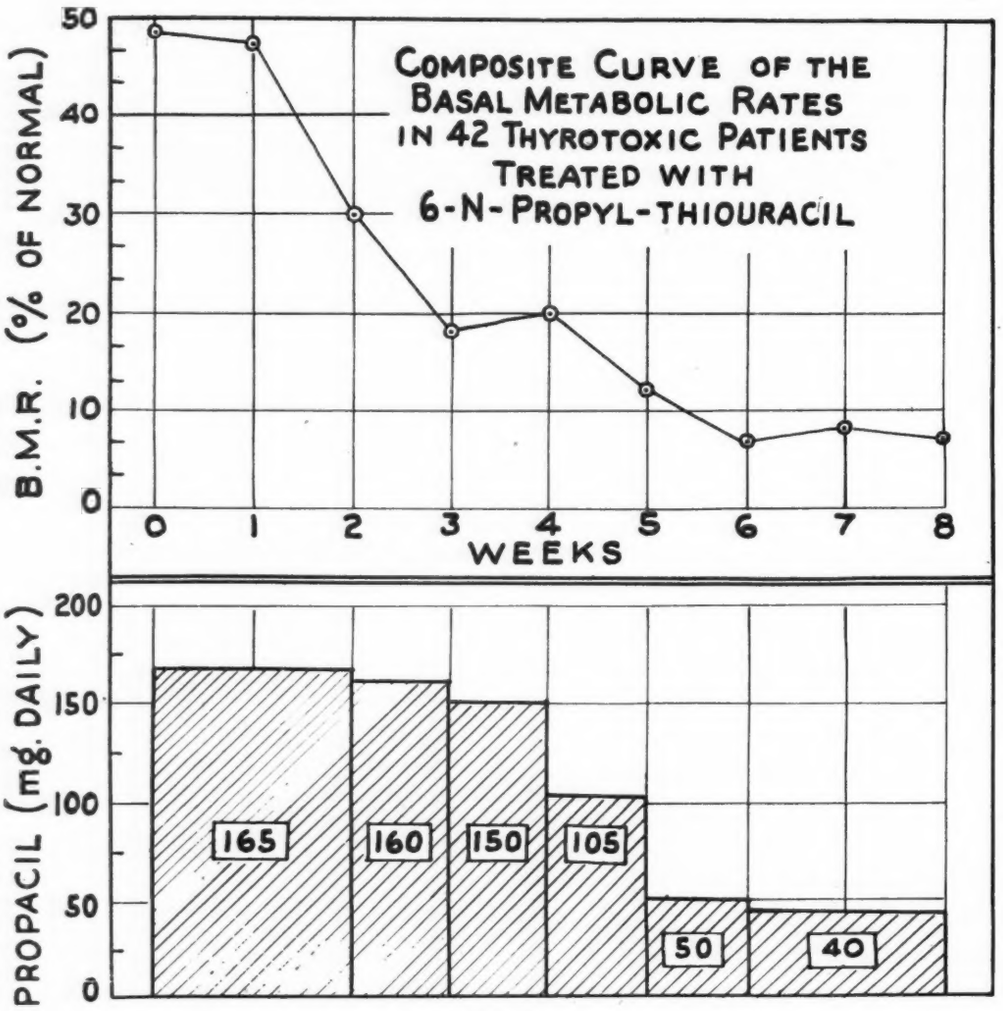


FIG. 1.

regimen of dosage indicated in Table III satisfactory. The largest doses mentioned,

TABLE III

SUGGESTED REGIMEN FOR EFFECTIVE ADMINISTRATION OF 6-N-PROPYLTHIOURACIL, COMPARED WITH 2-THIOURACIL

Period of Treatment (Days)	Thiouracil (Gm.)	Propacil (Gm.)
0–7	0.8*	0.250*
0–14	0.6	0.200
14-Control (14–49)	0.4	0.200
Maintenance	0.1–0.3	0.025–0.075

* These dosages may never be necessary. We have employed them when rapid "saturation" with the drug seem particularly important.

800 and 250 mg. daily for thiouracil and propacil respectively are always employed for a predetermined limited time only, which in any event should not exceed seven days. The method of slowly reducing the maintenance dose until the drug is omitted entirely is described below under Remissions (q.v.).

TOXICITY

In seventy-five patients, we have encountered but one reaction to propacil. This appeared in a forty-eight-year old woman with a so-called "postmenopausal type" of toxic nodular goiter. When first observed, she showed the majority of the toxic symptoms and signs originally described by Plummer. Various laboratory analyses were confirmatory, including a basal metabolic

rate of plus 43. Therapy with thiouracil had been attempted previously. On the fifth day of treatment with 0.6 Gm. daily of that drug she developed slight fever, and on the seventh day an accompanying generalized urticaria with the temperature up to 104.2°F. Five weeks later, she was given 50 mg. of propacil daily. On the seventh day after this was begun, she complained of generalized itching. By the ninth day, there was a return of the generalized rash and fever, the whole picture closely resembling that seen when she was treated with thiouracil, although less marked in degree.

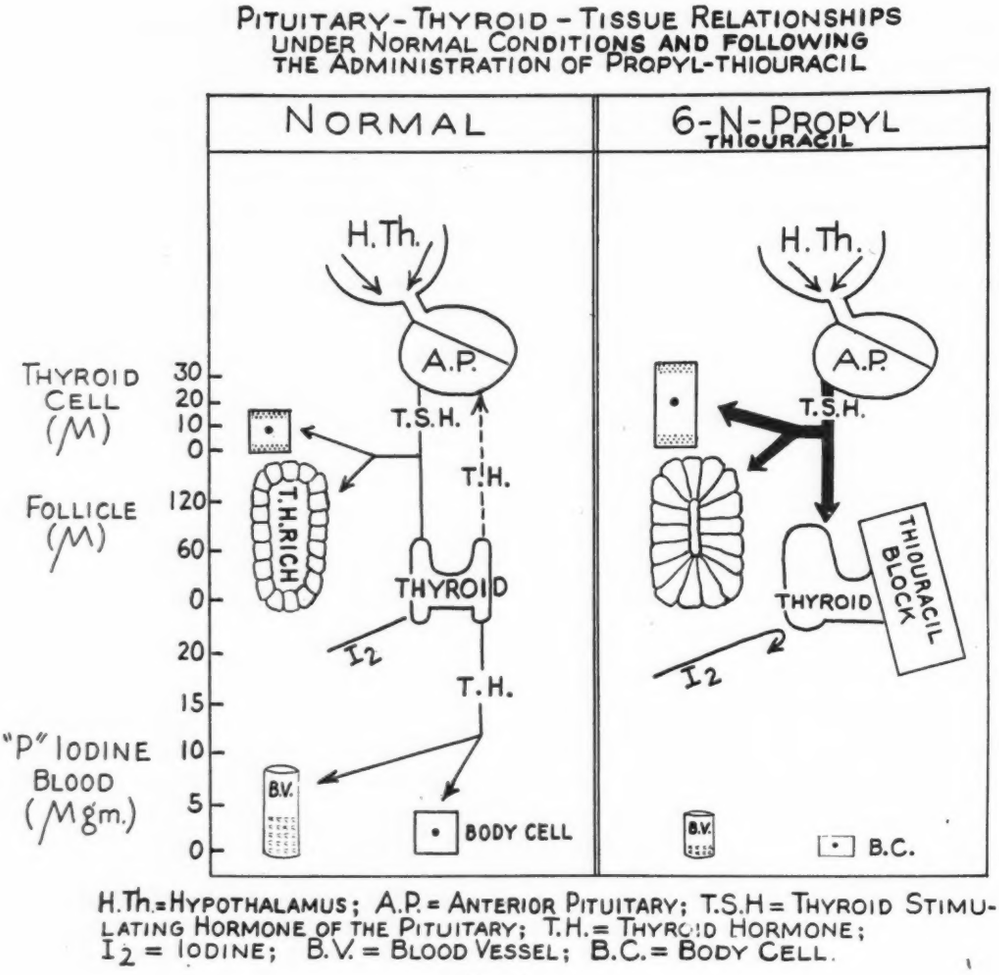
REMISSIONS

Much has been written about the tendency for the symptoms of hyperthyroidism to recur following cessation of treatment with thiouracil or one of its closely related derivatives. We believe this can be avoided in a large percentage of cases if attention is paid to certain details of treatment. After the patient has received a maintenance dose of propacil (Table III) for three months or more, it is our practice to decrease the dose by 25 mg. daily. At each succeeding monthly visit a similar reduction is made, until the patient has been maintained for one month on a single tablet of 25 mg. daily. Then, for an additional month, 25 mg. is administered every other day. If at the end of that time no symptoms or signs of thyrotoxicosis are apparent and the basal metabolic rate is normal, the drug is stopped. If at any time during the "reduction treatment" symptoms or signs of toxicity recur, the dosage is "stepped up" to the immediately preceding level and kept there until the clinical condition has remained satisfactory for three months. However, should signs of hypothyroidism occur earlier than this, the dose is accordingly reduced to restore the thyroid status to normal.

By following this regimen, we have been able to discontinue propylthiouracil in ten of the seventy-five patients without recurrence. However, since the drug has been available to us for slightly less than one year, we have been able to follow these "recovered" cases only for approximately four months following cessation of treatment. Inasmuch as all our patients were not started on the drug simultaneously at the beginning of the year, the above figure actually represents approximately 60 per cent of the group which has completed the regimen of dosage outlined above.

COMMENTS

The influence of thiouracil and its derivatives upon the pituitary-thyroid system has been explained by Means[18] according to the schematic representations of Galli-Mainini.[19] In accordance with this concept, the normal relationships between the hypothalmus, the pituitary, the thyroid and the fluids and tissues of the body may be diagrammatically indicated as in Figure 2. The alterations produced by propacil are shown for comparison in Figure 2. It will be seen that the action resembles that of thiouracil in every respect. The uptake of iodine by the thyroid gland is decreased. The formation of thyroid hormone is prevented. Therefore, the unopposed anterior pituitary secretes a greater amount of thyroid-stimulating hormone than normally. The thyroid cell changes to a high columnar type. The thyroid follicle enlarges. Its colloid is extruded and little if any is formed to take its place. In the face of this thyroid hyperplasia, there is a lack of thyroid hormone, with a consequent decrease in the protein-bound iodine of the blood and a decreased activity of the tissue cells. It must be emphasized that propacil appears not to effect a fundamental cure in thyrotoxicosis, any more than other commonly employed methods. Nevertheless, it "throws a block across the thyroid," preventing the manu-



H.Th.=Hypothalamus; A.P.=Anterior Pituitary; T.S.H=Thyroid Stimulating Hormone of the Pituitary; T.H.=Thyroid Hormone; I_2 = Iodine; B.V.=Blood Vessel; B.C.=Body Cell.

FIG. 2.

facture of thyroglobulin. Thus it breaks the vicious cycle of disturbed hypothalamic-pituitary-thyroid relationships which cause the toxic state. When the patient is no longer toxic, proper care can be given to the underlying mental and emotional problems without fear of further damage.

Within the limits just implied, the present studies and the clinical trials of other workers[9,14,15] indicate that propacil (6-n-propyl-thiouracil) will prove clinically useful in the management of all forms of thyrotoxicosis. It appears to be far superior to thiouracil. In animals the lethal dose (L.D.-50) is slightly less than that of thiouracil and its goitrogenic activity approximately eleven times that of thiouracil. In earlier studies in the human being, propacil was thought to be approximately five times as effective as thiouracil[9] but such an estimate requires downward revision in the light of the present and other data;[14,15] it appears to be from two to three times as effective as thiouracil, weight for weight.

Moreover, in any range of effective dosage, propacil appears to be far less toxic than thiouracil. Astwood and VanderLaan[14] report no ill effects in their first 100 patients. McCullagh and his associates[15] discontinued the drug in one of 110 patients treated because "mild sore throat and a fall in leukocyte count followed repeated trials." Both the groups of workers just mentioned have used propacil successfully in patients that developed severe reactions to other thiouracil-related compounds.

One patient in the present series, already shown to be sensitive to thiouracil, also developed a reaction to propacil. Untoward effects were not encountered in any other patient. Such low toxicity contrasts sharply with thiouracil, under treatment with which from 12 per cent to 13.1 per cent of all patients developed some untoward manifestation[10,11,13] and 2.5 per cent exhibited the more serious febrile or granulocytopenic reactions. Death from granulocytopenia has been reported in about 0.5 per cent of patients treated with thiouracil.[10] Neither complete agranulocytosis nor death has yet been reported due to propacil. Since such a reaction is probably due to true toxicity rather than to drug hypersensitivity, it should occur rarely if at all in any range of dosage of propacil necessary to achieve an optimum therapeutic effect.

In conclusion, we believe that propacil is a relatively safe drug for the management of thyrotoxicosis of all types. It should replace operative interference except in those instances in which local pressure symptoms occur or unsightliness of the neck makes elective surgery highly desirable.

SUMMARY

1. Seventy-five patients with thyrotoxicosis have been successfully controlled by the use of 6-n-propylthiouracil (propacil) in initial doses varying from 75 to 250 mg. and maintenance doses of from 25 to 75 mg.

2. One severe febrile reaction was encountered in a patient who had previously developed a similar response to thiouracil.

3. Ten of the seventy-five patients have had no recurrence of thyrotoxic symptoms four months after discontinuing the drug.

4. It is concluded that 6-n-propylthiouracil is a safe and effective drug for the management of all forms of hyperthyroidism.

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Thiouracil: Remission or Relapse of Hyperthyroidism after Discontinuing Its Use*

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THIOURACIL has been used in the treatment of hyperthyroidism for a sufficient length of time so that its place in the therapy of this disease should now be evaluated. All investigators agree that hyperthyroidism, irrespective of type or severity, can, by an adequate daily dose of thiouracil, be brought under complete control. Thirouacil has proved to be of great value in preparing patients[1,2] with severe hyperthyroidism for thyroidectomy since multiple-stage operations, postoperative reactions with morbidity and mortality can be completely prevented in these patients who are properly treated. Thiouracil has been used for long-continued maintenance treatment of hyperthyroidism but this type of therapy entails close observation because of the possibility of toxic reaction. As regressive changes are not produced in the hyperplastic thyroid gland following thiouracil administration, permanent cure of hyperthyroidism is not to be expected. The clinical course after withdrawal of treatment of patients whose disease has been controlled by thiouracil is the subject of this report.

Astwood[3] has reported that after controlling hyperthyroidism for a prolonged period, from six to nine months, thiouracil could then be discontinued and in a high percentage of patients a remission would be sustained. He emphasized prolonged treatment as the prerequisite for obtaining a prolonged remission. Reports by other observers[3–13] (Table 1) vary widely regarding remissions and relapses after withdrawal of treatment. Prolonged remissions were reported by various authors to occur in from 20 to 82 per cent and relapses in from 18 to 80 per cent of the patients treated. Although little significance can be given to the wide variation found in the smaller series of cases

TABLE 1
REMISSIONS AND RELAPSES AFTER WITHDRAWAL OF THIOURACIL

Authors	No. of Cases	Remission		Relapse	
		No.	Per Cent	No.	Per Cent
Astwood	18	9	50	9	50
Rose and McConnell	21	8	38	13	62
Gabrilove-Kert-Soffer	5	4	80	1	20
Reveno	5	1	20	4	80
Palmer	10	5	50	5	50
McGavack-Gerl-Morton-Vogel-Schwimmer	"ability to discontinue the drug in the average case without recurrence of symptoms has varied widely"				
				2	
Watson	6	5	83	1	17
Barr, Shorr	47	36	77	11	23
Fishberg, Vorzimer	41	16	39	25	61
Williams et al	100	49	49	51	51
Total cases	253	133	54	120	46

* From the Lahey Clinic. Read before the Section on Experimental Medicine and Therapeutics, American Medical Association, San Francisco, July 3, 1946.

reported, cases in the larger series were found to vary just as widely. Those authors reporting forty or more hyperthyroid patients found remissions in from 39 to 70 per cent and relapses in from 23 to 61 per cent. Most authors reported no relationship between the duration of treatment and the development of remission or relapse. McGavack[8] and his coworkers stated that the "ability to discontinue the drug in the average patient without recurrence of symptoms varied widely." Barr and Shorr[10] noted that the severest cases and particularly those patients who had recurrences after previous thyroidectomy had relapse almost as soon as treatment was stopped. Reveno's[6] studies led him to conclude that a prediction could not be made as to when and in which cases the drug may be stopped without relapses. He recommended thiouracil only for maintenance treatment.

The most complete work on this subject is that by Williams[12] who studied 100 hyperthyroid patients who had received prolonged thiouracil treatment. Of these, forty-nine have gone three to twenty-one months without treatment and have not had relapse. Fifty-one patients had relapse of hyperthyroidism after two weeks to five months. Of these, 66 per cent had relapse in one month. Williams[12] noted that the age of the patient, duration or type of hyperthyroidism did not influence relapse. Male patients had relapse of hyperthyroidism more often than female patients. The longer treatment was carried out, the lower the initial basal metabolic rate and the smaller the thyroid gland, the greater was the chance of remission. Patients with thyroid glands three to four times normal size did have sustained remissions, however, as did patients with initially high basal metabolic rates. There was no relation of relapse or remission to the dosage of thiouracil given or the speed of improvement. Of the total 253 cases reported by nine authors, 53.9 per cent remained in remission and 46 per cent had relapse.

Experience with twenty-one patients who have been observed for as long as two and a half years from the standpoint of remission or relapse after withdrawal of thiouracil is the basis of this report. All of the patients had primary hyperthyroidism; eleven had recurrent hyperthyroidism. Treatment with thiouracil was begun in all patients in the dose of 0.6 Gm. a day and in some patients it was continued at this dose up to the time of its withdrawal. In other patients the dose of thiouracil was gradually decreased as improvement was noted. Thiouracil was administered for as long as twenty months in one case; the shortest treatment was two months. After withdrawal of treatment, eight patients remained in remission and thirteen patients suffered relapse of hyperthyroidism.

TABLE II
REMISSION AFTER WITHDRAWAL OF THIOURACIL

Case	Age and Sex	Duration of Hyperthyroidism	Duration of Treatment	Duration of Remission	Initial BMR	Last BMR	Thyroid Enlargement
1	23 F	3 mo.	10 mo.	14 mo.	+27	−1	Slight
2	58 F	1 yr.	6 wk.	19 mo.	+25	+7	Slight
3	73 F	7 mo.	11 mo.	13 mo.	+19	+8	Small remnant
4	20 F	1 yr.	5 mo.	17 mo.	+28	+8	Slight
5	30 F	6 mo.	14 mo.	3 mo.	+35	+5	Slight
6	60 F	6 mo.	1 mo.	23 mo.	+17	−1	Slight
7	12 F	3 yr.	21 mo.	3 mo.	+23	+2	Small remnant
8	18 F	3 mo.	7 mo.	14 mo.	+27	+4	Small remnant

The eight patients (Fig. 1, Table II) who have remained in remission include five with initial primary hyperthyroidism and three with recurrent primary hyperthyroidism. All were females with a wide range of ages, twelve to seventy-three years. These patients had hyperthyroidism from three months to three years; five had the disease for seven months or less, the average dura-

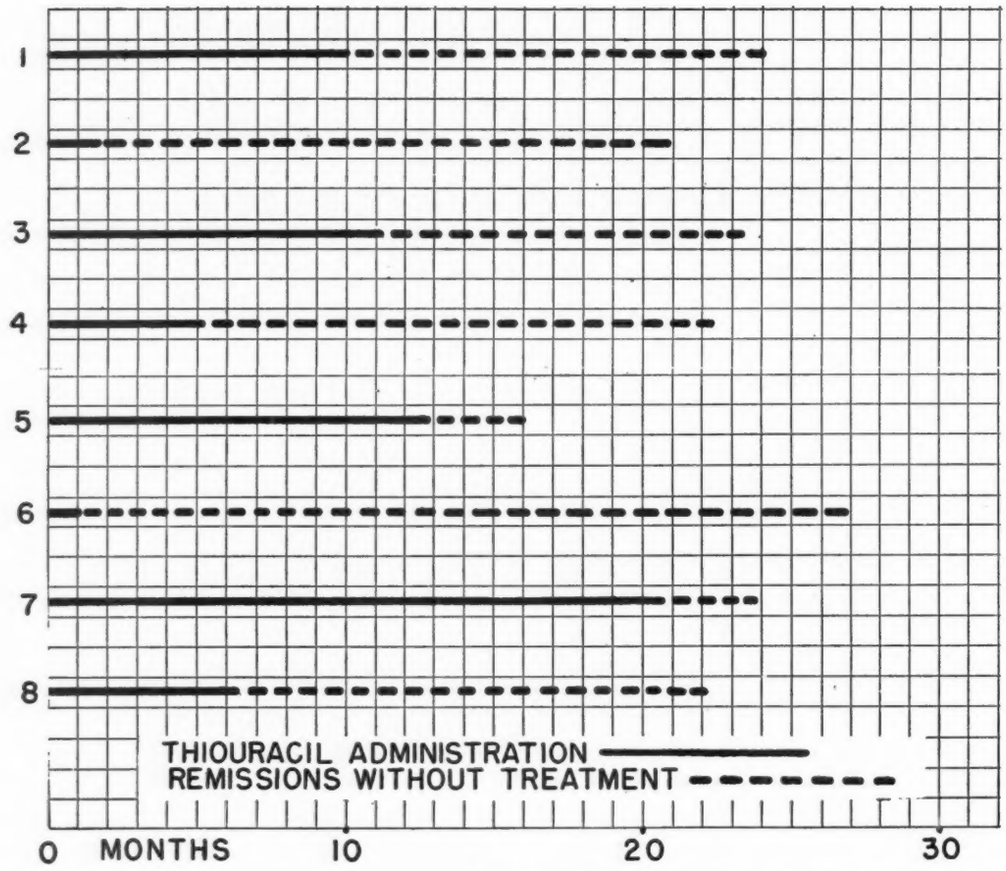

FIG. 1. Duration of thiouracil therapy in eight hyperthyroid patients and length of remission after withdrawal of treatment.

tion being eleven months. Thiouracil was administered for one to twenty-one months, the average time being nine months. The basal metabolic rate before treatment ranged from +17 to +35, the average being +25. The thyroid gland of all these patients was only slightly enlarged or the remnants were small. Remissions of nine months' duration or more occurred in six patients and of three months' duration in two patients. These latter two patients may still have a relapse. The average duration of remission is thirteen months.

The thirteen patients (Fig. 2, Table III) having relapse after withdrawal of thiouracil include nine patients with recurrent primary hyperthyroidism and four patients with initial primary hyperthyroidism. The age of these patients varied from twenty-seven to fifty-five years, the average being forty years. Nine were female patients and four were males. Hyperthyroidism had been present for one month to five years, an average of fourteen months. Eight patients had hyperthyroidism for six months or less. Treatment was given for a period of two and a half to eighteen months, the average being eight months. The average basal metabolic rate was +30; 7 had a basal rate over +30. The thyroid glands or remnants were medium to large in size in twelve of the patients in this group; one patient (Case 13) had only slight enlargement of the thyroid. Relapse occurred in all from one to twelve months after withdrawal of thiouracil, the average being three and one half months; eleven had a relapse in one to six months. In two of the patients treated for fourteen to eighteen months, relapse occurred after two months. Of the thirteen patients having

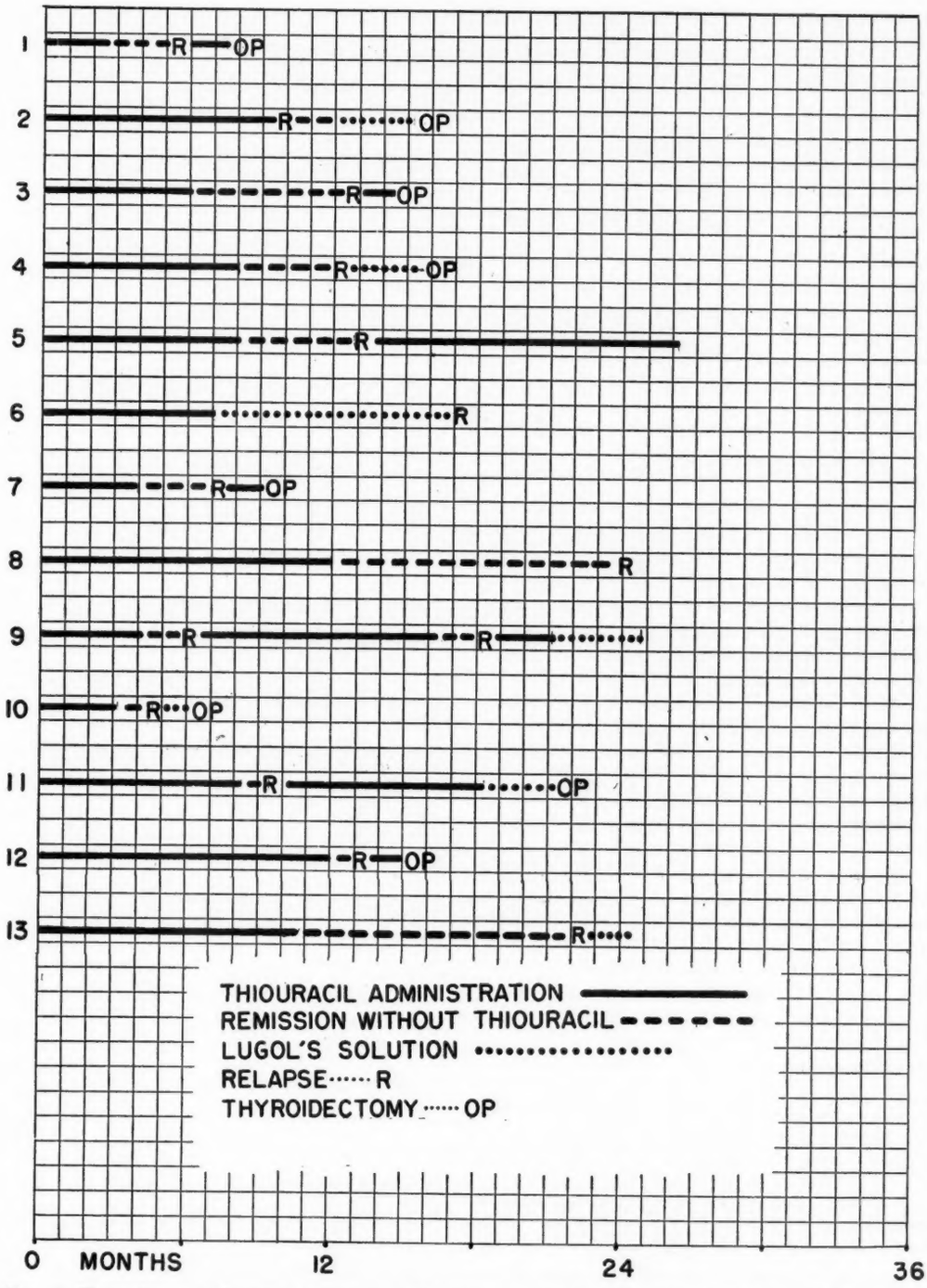

FIG. 2. Duration of thiouracil therapy in thirteen hyperthyroid patients, with remission and then relapse after withdrawal of treatment.

relapse, eight have had thyroidectomy; one is being maintained on thiouracil and three are receiving Lugol's solution.

A comparison of the two groups of patients (Table IV), those having sustained remission and those having relapse, reveals that the patients who had relapses were slightly older and had hyperthyroidism of slightly longer duration; the difference, however, was not significant. The duration of treatment with thiouracil was approximately the same in the two groups. Male

TABLE III

RELAPSE AFTER WITHDRAWAL OF THIOURACIL

Case	Age and Sex	Duration of Hyperthyroidism	Duration of Treatment	Onset of Relapse	Initial BMR	Thyroid Enlargement	Thyroid Tissue Removed at Thyroidectomy	
							Weight	Size, cm.
1	49 M	6 mo.	2 mo.	2 mo.	+45	Moderate	40 Gm.	7 × 3.5 × 2 6 × 3 × 1.5
2	55 M	4 yr.	10 mo.	2 mo.	+46	Moderate	50 Gm.	6 × 3 × 2 6 × 3 × 2
3	47 F	6 mo.	6 mo.	6 mo.	+45	Medium remnant	10 Gm.	3 × 2 × 1 2 × 2 × 1
4	33 F	3 mo.	8 mo.	4 mo.	+38	Medium remnant	15 Gm.	7 × 2 × 2
5	48 F	2 mo.	8 mo.	4 mo.	+26	Medium remnant		
6	42 F	6 mo.	7 mo.	1 mo.	+18	Medium remnant		
7	31 M	2 yr.	18 mo.	2 mo.	+41	Large	115 Gm.	9 × 5 × 3 10 × 5 × 2.5
8	31 F	1 yr.	12 mo.	12 mo.	+30	Medium remnant		
9	54 F	2 mo.	4 mo.	2 mo.	+3	Medium remnant		
10	42 F	13 mo.	3 mo.	1 mo.	+14	Medium remnant	10 Gm.	
11	27 F	1 mo.	8 mo.	1 mo.	+17	Medium remnant	12 Gm.	4 × 2 × 2 3 × 2 × 1.5
12	37 M	5 yr.	14 mo.	1 mo.	+35	Large remnant	55 Gm.	8 × 5 × 3 4 × 3 × 2 4 × 3 × 2
13	27 F	3 mo.	10½ mo.	11 mo.	+13	Small		

TABLE IV

COMPARISON OF RELAPSE AND REMISSION GROUPS

	No. of Cases	Av. Age, Yr.	Sex	Duration of Hyperthyroidism	Duration of Treatment	Initial BMR	Thyroid Enlargement	Duration of Remission
Group obtaining remission.	8	36	F	3 mo. to 3 yr.	1 mo. to 21 mo.	+17 to +35	Slight	
				Av. 11 mo.	Av. 9 mo.	Av. +25	Small remnants	Av. 13 mo.
Group suffering relapse.	13	40	9F 4M	1 mo. to 5 yr.	2 mo. to 18 mo.	+3 to +46	Medium to large	1 mo. to 12 mo.
				Av. 14 mo.	Av. 8 mo.	Av. +30	Large remnants	Av. 3½ mo.

patients tended to have relapse of hyperthyroidism more than did female patients. Seventy per cent of the patients having relapse had recurrent hyperthyroidism, as against 37 per cent in the group remaining in remission. Most of the relapsing group had higher basal metabolic rates and moderate to large thyroid glands or remnants. Eight of the patients having relapse had subtotal thyroidectomy or removal of thyroid remnants. The amount of the thyroid tissue removed at operation varied in weight from 40 to 115 Gm., the remnants from 10 to 12 Gm., which represents a substantial amount of thyroid tissue since the normal thyroid gland weighs from 16 to 24 Gm.

SUMMARY

Patients with primary hyperthyroidism may have a prolonged remission from hyperthyroidism after withdrawal of thiouracil. Of the twenty-one patients observed, eight (32 per cent) remained in remission and thirteen (62 per cent) had a relapse. The duration of thiouracil administration after restoration of the basal metabolic rate to normal was not a factor in determining the duration of remission since prolonged remissions occurred after short therapy and prompt relapse was observed after long treatment.

Clinical experience indicates that patients with mild primary hyperthyroidism may occasionally have a prolonged remission either spontaneously or following brief iodine therapy, and that such a remission may last many years. Therefore, thiouracil in a sense might be classified with iodine in its power to cause a remission in mild hyperthyroidism and if the eight patients now in remission are observed sufficiently long, all can be expected to have a relapse.

Relapse of hyperthyroidism after withdrawal of thiouracil can be expected to occur in one to two months and not later than six months after withdrawal of treatment in patients having large thyroid glands or large recurrent remnants, in whom the basal metabolic rate is in the higher range. The basic etiologic factor which causes hyperthyroidism, which at present cannot be assayed, is, of course, the determining cause of recurrence or remission, and this factor is not affected by thiouracil therapy.

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Review

Brucellosis and Infection Caused by Three Species of Brucella*

Clinical, Laboratory and Epidemiological Observations

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BRUCELLOSIS of man or undulant (Malta) fever is with few exceptions a disease of sporadic occurrence. Ordinarily, very few cases are reported from an average rural county during the course of an entire year. Exceptions to this rule are (1) the greatly increased morbidity among persons whose occupation brings them into close contact with animals at the time of slaughter and (2) the certainty of multiple cases when the more virulent porcine species of brucella contaminates a raw milk supply. Illness from this disease is always traceable to infection in animals and is not known to be communicable from person to person. Direct contact with infected animals and the use of raw dairy products from infected dairy cows provide the main avenues of transmission to man. The attack rate is highest among individuals (such as packing house employees, male farm workers, veterinarians), whose occupation brings them into direct contact with infected animals, and lowest among urban residents who use none but pasteurized dairy products and who give no history of handling livestock.

During the fourteen-year period 1930 to 1943, reported cases of brucellosis of man in the United States totalled 36,513, an average annual morbidity rate of but two per 100,000 population. As might be expected, the disease shows relatively higher incidence in hog,† cattle and sheep raising areas, notably the West North Central and West South Central States. (Table 1.)

TABLE 1

BRUCELLOSIS IN THE UNITED STATES, 1930–1943

Morbidity for the fourteen-year period as reported from various sections of the country, and average annual rates per 100,000 population

Area	Population (1940 Census)	1930–1943 Total Cases Reported	Average Annual Cases	Annual Rate Per 100,000
New England	8,437,290	2,458	175.5	2.08
Middle Atlantic	27,539,487	4,971	355.1	1.29
East North Central	26,626,342	6,828	487.7	1.83
West North Central	13,516,990	7,107	507.6	3.76
South Atlantic	17,160,060	2,929	209.1	1.22
East South Central	10,778,225	1,479	105.6	0.98
West South Central	13,064,525	6,105	436.1	3.34
Mountain	4,150,003	1,157	82.6	1.99
Pacific	9,733,262	3,479	248.4	2.56
U. S. A. Total	131,006,184	36,513	2608.0	1.99

Data presented in Table 1 were compiled from totals of reported cases in each state, supplied through courtesy of state health officers of the forty-eight states.

Considering individual states, the lowest rate for the twelve-year period 1930 to

† The swine population in Iowa was approximately 20,000,000 in the peak years 1943 and 1944, estimated as 20 per cent higher than during pre-war years. (From data furnished through courtesy of C. C. Franks, D. V. M., State Veterinarian, Iowa State Department of Agriculture.)

* From the Division of Preventable Diseases, Iowa State Dept. of Health and the Iowa State Hygienic Laboratory.

1941, namely 0.4 per 100,000, was in North Carolina, significantly the only state at the time to be accredited in measures for the eradication of brucellosis in dairy cattle. Considering the reported occurrence of cases, hogs are apparently a minor source of infection in that state.

The annual morbidity rate from brucellosis in Iowa for the five-year prewar period 1935 to 1939 was 5.31 per 100,000. Due largely to increased pork and livestock production, the rate increased to 13.00 per 100,000 during the five years 1940 to 1944, corresponding in general to the period of World War II. The number of positive agglutination reports as notified from the State Hygienic Laboratory is considerably greater than that of officially reported cases. With complete reporting based on positive agglutination in diagnostic dilutions, rates per 100,000 might be nearly twice as high as here presented.

EPIDEMIOLOGIC FACTORS AND FINDINGS

Age, Sex and Seasonal Prevalence. Out of a total of 2,082 brucellosis case reports, completed through interest and courtesy of Iowa physicians during the twelve-year period 1933 to 1944, male patients numbered 1,639 and females 443, a male to female ratio of about five to one. Among patients under nine and over seventy years of age, the disease occurs as frequently in females as in males, indicating (1) equal susceptibility in the two sexes and (2) probable exposure through unpasteurized dairy products. The preponderance of males over females in the teen-age group and in the adult decades below seventy, emphasizes the major rôle played by direct contact in causing the marked difference in attack-rate in the two sexes. Although brucellosis is with us always, every month of the year, more cases have onset of symptoms in June, July and August (probably following contact with animals during the farrowing and calving season) than in any other three-month period.

Occupation and Residence. Analysis of 1,378 case reports contributed by Iowa physicians during the five-year period 1939 to 1943, showed an average annual rate of fourteen cases per 100,000 population in the farm group, compared with nine cases per 100,000 in cities and towns under 2,500 and five cases per 100,000 in cities over 2,500, exclusive of packing house workers. (Table II.)

The specific rate among packing house employees for the same period was 245 per 100,000, about fifty times as high as the rate among urban residents who do not come into direct contact with livestock.

INFECTION CAUSED BY THE BOVINE ORGANISM, BRUCELLA ABORTUS

Discovery of Brucella abortus ("bovis" preferred by the late Prof. Wm. H. Holmes) as the usual causative agent of brucellosis in the cow, was announced by the veterinarian Bang in 1896. The first case of brucellosis of man or undulant fever with probable origin in the United States, was reported by Craig[1] in 1905. Schroeder and Cotton[2] in 1913 demonstrated the presence of this organism in the milk of cows infected with brucellosis or Bang's disease. Alice Evans,[3] recently retired as Senior Bacteriologist, U. S. Public Health Service, made a distinct contribution in 1918 in showing that there was no demonstrable difference between brucella as recovered from a patient and a culture of Brucella abortus from an infected cow. Miss Evans also stated that human illness might result from exposure to infection in animals. A case of brucellosis of man or undulant fever which occurred in Baltimore in 1922, was reported by Keefer.[4] Acken[5] reported a case from New York in 1926 and Carpenter

TABLE II

BRUCELLOSIS IN IOWA 1939–1943

Number of Cases in Urban and Rural Areas and Rates per 100,000 Population Based on 1,378 Case Reports Completed through Courtesy of Iowa Physicians

Year	Rural Areas: Farm Group[a]		Rural Areas: Cities, Towns under 2500[b]		Urban Areas: Cities over 2500[d]				State Totals[e]	
	Farm Residents		All Others		Packing House Workers[c]		All Others		All Cases	
	No.	Rate per 100M	No.	Rate per 100M	No.	Spec. Morbid Rate	No.	Rate per 100M	No.	Rate per 100M
1939	80	8.7	37	6.9	19	95.0	37	3.4	173	6.8
1940	107	11.7	39	7.3	67	335.0	48	4.4	261	10.3
1941	135	14.7	37	6.9	35	175.0	59	5.4	266	10.5
1942	150	15.5	53	9.9	62	310.0	65	6.0	330	13.0
1943	181	19.7	68	12.7	54	270.0	45	4.2	348	13.7
Totals 1939–1943	653		234		237		254		1378	
Avg. ann. cases	131		47		47		51		276	
Avg. rate per 100M		14.3		8.7		245.0		4.7		10.9

[a] 916,768 rural, farm population—Census, 1940.
[b] 537,269 rural, non-farm population—Census, 1940.
[c] 20,000 packing house workers (estimated total).
[d] 1,084,231 urban population—Census, 1940.
[e] 2,538,268, total population—Census, 1940.

and Merrian[6] reported two more cases in that state in the same year. In Iowa, clinical diagnosis of the first case of brucellosis was made by Woodward[7] in December, 1926. Subsequent laboratory and epidemiological study of the disease in Iowa and the United States was made by Hardy[8] and associates.[9]

Brucellosis due to Br. abortus is usually of sporadic occurrence, regardless of whether illness results from unpasteurized dairy products or from direct contact with infected cows. It is highly probable that situations similar to that described in the following paragraphs occur from time to time in communities throughout the country.

1. *Clinical Cases in Greenfield.* In September, 1942, and during July and August, 1943, three cases of brucellosis occurred in Greenfield (population 1,869), county seat of Adair County, Iowa. Those ill were C. W., fifty-two year old male patient of Wm. F. Crew, M.D.; P. C., fifty-six year old male and A. H., thirty-two year old male, patients attended by E. O. Reynolds, M.D. The diagnosis was made on the basis of symptomatology and the presence of agglutinins in diagnostic dilution (1:1280,

1:320 and 1:160, respectively) in the blood serum of these individuals. None of the patients had recently been in direct contact with farm animals but all had used raw milk as distributed by a local, the R dairy.

Since many families in the city had used the same milk as the three patients, an agglutination and skin test survey was carried out in February, 1944, in cooperation with local physicians, city and school officials, to obtain further information as to the extent of infection.

2. *Results of Agglutination Tests.* Agglutination tests were performed at the State Hygienic Laboratory on the blood serum of 232 persons (mostly children in upper grades, high school students and some adults). The serum of twenty-three individuals (10 per cent) showed positive agglutination in dilutions from 1:40 to 1:2560 as follows: seven in 1:40, five in 1:80, five in 1:160, four in 1:320, 1 in 1:1280 and 1 in 1:2560. Blood cultures were taken from six persons whose agglutination tests were positive in dilutions 1:160 and above; efforts to isolate Br. abortus through a period of a month of incubation and transfer of cultures were unsuccessful.

3. *Latent, Subclinical Infection.* Of the group of twenty-three persons with positive agglutination as listed, two married women, thirty-one and thirty-five years of age respectively and a school girl, age eleven, gave the history of recent mild illness not previously recognized as brucellosis, lasting several weeks and characterized by fever, chills or chilliness, loss of weight, tired feeling and in the case of the child, also night sweats and pallor. The other twenty were apparently well, showing evidence of latent infection but without clinical manifestations. None of the twenty has since been reported as having suffered illness.

4. *Results of Intradermal Tests.* Intradermal tests were administered with brucellergen* using 0.1 cc. of a 1:12,000 dilution. Among 248 tested, 48 or 20 per cent showed positive reaction in forty-eight hours, with erythema and edema varying from 15 to 90 mm. in diameter. Of 114 persons having used milk from the R dairy (suspected as the source of infection), 25 or 22 per cent had positive skin tests. Of 134 individuals using milk from their own cows or other sources, 23 or 16 per cent showed positive skin reactions. The fact that 16 per cent of apparently normal individuals in the survey showed an allergic response following the use of brucellergen, is evidence of the unreliability of the intradermal test alone, to establish a clinical diagnosis of brucellosis.

5. *Isolation of Brucella from Milk.* Cream from the R dairy was inoculated into two guinea pigs March 2, 1944, at the State Hygienic Laboratory. On April 17th, the spleen and liver of these animals as reported by one of the authors (I. H. B.), were two to three times normal size and irregularly roughened by 1 to 3 mm. abscesses or granulomata. Serum of the guinea pigs agglutinated brucella antigen (1:640 and 1:1280) and Br. abortus was isolated from the organs of both.

6. *Milk Now Pasteurized.* It should be added that through efforts of the milk sanitarian of the State Department of Health and of informed city officials, together with results of the survey, two pasteurizing plants were promptly installed.

INFECTION CAUSED BY THE PORCINE ORGANISM, BRUCELLA SUIS

Br. suis was first isolated from fetuses of infected swine by the veterinarian Traum, in 1914.

Infection Resulting from Direct Contact. Former field investigation of brucellosis cases in Iowa in cooperation with attending

* Furnished through courtesy of Michigan State College by I. Forest Huddleson, D. V. M.

physicians and the United States Public Health Service, revealed that many of the patients gave the history prior to illness, of using dairy products only sparingly, but of having had direct contact with hogs; some of the sows had lost litters and were later found to react to the agglutination test for brucellosis. Blood cultures from a number of the patients yielded a strain of brucella that proved, when tested by Huddleson's[10] dye-method of differentiation, to be Brucella suis.

As suggested by Hardy,[11] direct contact with hogs must be considered a major factor to account for the relatively high incidence of brucellosis in the midwestern states of this country (see rates per 100,000 in Table 1). Cases resulting from direct contact, whether with infected hogs or cows, are usually of sporadic and accidental occurrence; such instances in the aggregate, probably exceed in number those traceable to contaminated dairy products. Cases of brucellosis affecting farm workers result from varied sources of infection on individual farms. The extent of brucella infection is more readily determined in the meat packing industry where many are exposed to an enormous concentration of animals, some of which are known to harbor brucella. Surveys conducted in Iowa in past years have shown the incidence of active and latent infection among apparently healthy workers as determined by positive agglutination reactions (1:40 or above), on one occasion to be 18.2 per cent (240 tested)[12] and 10.8 per cent of 251 tested at a later time.[13]

The importance of swine brucellosis is demonstrated in a report by McNutt[14] who examined 1,547 hogs by the rapid agglutination method and found 3 per cent of the animals infected. He isolated Br. suis from 14 or 41 per cent of thirty-four reacting animals; organisms were cultured from the spleen, liver, uterus and lymph nodes.

Infection Resulting from Unpasteurized Dairy Products. From time to time, a raw milk supply becomes contaminated with Br. suis, brought about by hogs sharing the same lot with dairy cows. When infection is transmitted from infected hogs to the udder of one or more dairy cows, multiple cases of brucellosis are certain to result, probably because Br. suis is more invasive, prone to produce a more severe infection than that from Br. abortus. Three epidemics of this nature have been investigated in Iowa under auspices of the State Department of Health during the past thirteen years. Report of the first epidemic was made by Beattie and Rice,[15] of the second by Borts and associates.[16] Clinical and epidemiologic findings in a third outbreak, are summarized as follows:

1. *Multiple Clinical Cases in Bennett.* During November, December and January, 1942 and 1943, an outbreak of brucellosis caused by Br. suis occurred at Bennett (population 352), Cedar County, Iowa. Six cases of the disease were diagnosed by local physicians, based on positive agglutination reactions on blood serum, ranging from 1:320 to 1:1280. In February, 1943, an agglutination and skin test survey was carried out at Bennett, in cooperation with school and town officials, L. E. Bees, M.D., A. R. Stephenson, D.V.M. and D. M. Harris, M.D., then Director of District Health Service No. 8.

2. *Results of Agglutination Tests; Latent Infection.* Agglutination tests were performed on the serum of 119, mostly consolidated school students but including some adults in the community. Twelve persons (10 per cent) showed positive agglutination in dilutions 1:40 to 1:2560 as follows: two in 1:40, five in 1:160, one in 1:320, three in 1:640 and one in 1:2560. Of the twelve, one boy had missed school several days due to a "cold," with cough and slight fever. The remaining eleven gave

no history of illness, showing latent infection without clinical symptoms. Three of the children with latent infection likewise had a bacteremia, Br. suis being isolated from blood cultures. None of the eleven has been reported as having illness caused by brucellosis since the time of the survey.

3. *Results of Skin Tests.* Skin tests were performed on 112 persons, with brucellergen furnished through courtesy of Dr. I. F. Huddleson, D.V.M., of Michigan State College. Among fifty-one who had used contaminated milk from the M dairy (Br. suis was isolated from the milk), 24 or 47 per cent showed erythema and edema in forty-eight hours. Out of sixty-one using milk from their own cows or from other sources, 15 or 25 per cent showed positive skin reactions. Eleven of the twelve with positive agglutination findings also showed allergic response to brucellergen.

INFECTION CAUSED BY THE CAPRINE STRAIN, BRUCELLA MELITENSIS

It was the British physician, David Bruce,[17] who in 1887 was the first to isolate the causative organism from the blood of patients on the island of Malta who suffered from a febrile disease known as Mediterranean or Malta fever. All cases were traced to goats as the source of infection. In 1911, patients of the same disease in Texas and likewise with source in goats, were investigated and reported by Ferenbaugh,[18] and Gentry and Ferenbaugh.[19] Yount and Looney[20] in 1912 reported five patients with Brucellosis melitensis in Arizona and in 1922 Lake[21] diagnosed and reported thirty-five cases of this type of the disease in the same state. In 1935, Meyer and Eddie[22] reported results of a survey of Br. melitensis infection in goats in the southwest. Evans[23] in 1937 reported the distribution of Br. melitensis in the United States, including recognition of human cases in Texas, North Carolina and Kansas. Of 150 strains of Brucella isolated from patients hospitalized at General Hospital, Mexico City (1938 to 1941) and reported by Castaneda, Tovar and Velez[24] in 1942, strains numbering 143 (95 per cent) were Br. melitensis.

Br. suis and Br. abortus were for years believed to be the only two species of brucella to occur in Iowa. Although Br. melitensis was isolated from the blood of a patient hospitalized in Iowa in 1930,[25] the man was a Mexican and the melitensis infection was apparently acquired in Mexico, since onset of illness developed but a few days after the patient had left his native country.

Br. melitensis is now known to be endemic in Iowa. Between December, 1943 and July, 1946, the melitensis strain was recovered from the blood of forty patients in Iowa. Since July, 1944, one of the authors (I. H. B.)[26] has employed a tryptose broth medium (with technic modified from that of Bohls and Schuhardt), which renders possible the isolation of brucella strains not alone from blood cultures but also from the blood clot contained in the specimen (aseptic precautions essential) which the physician forwards to the laboratory for the agglutination test.

In a series of twenty Br. melitensis cases investigated in Iowa during 1945, ten were packing house workers; the remaining ten were farm workers or visitors on farms. Only seven of the twenty patients had been in contact with sheep preceding illness, and none with goats. Twelve, or 60 per cent of the patients were in direct contact with hogs only, prior to onset of illness gave no history of contact with sheep. Recovery of Br. melitensis from tissues of hogs taken from a farm in Iowa has recently been reported by Jordan, Borts and McNutt.[27] Milk cows on the farm concerned failed to react to the brucella agglutination tests and brucella was not isolated from the milk following cultural and guinea pig inoculation.

In April 1946, McNutt isolated another strain of Br. melitensis from tissues of a hog belonging to a farmer whose wife developed brucellosis. Shortly before onset of symptoms, the patient had handled newborn pigs. Her serum showed (1:1280) agglutination and the blood clot yielded Br. melitensis.

ISOLATION OF BRUCELLA FROM PATIENTS

During the period from September, 1927 to December 1, 1945, brucella strains totalling 358 were isolated at the State Hygienic Laboratory from the blood and tissues of brucellosis patients in Iowa. Of these strains, 238 (66 per cent) were Br. suis, 88 (25 per cent) were Br. abortus and 32 (9 per cent) were Br. melitensis.

Damon,[28] former Director of Laboratories, Alabama State Department of Health recovered ninety-one brucella strains during the five-year period 1939 to 1943. Sixty-nine (76 per cent) of the strains were Br. suis, twenty-one (23 per cent) Br. abortus and one (1 per cent) untyped.

Similarity in blood culture findings as compiled in Alabama and Iowa serves to emphasize the importance of hogs as a relatively frequent source of human infection not only in the corn belt but also in the South.

Pathology. Mortality from brucellosis as a direct cause of death does not usually exceed 2 or 3 per cent. Forbus[29] describes the pathological findings in three types of brucellosis: (1) acute, septicemic, (2) subacute, focal or localized and (3) chronic lymphogranulomatous.

According to Forbus: "The septicemic case shows little that is specific of brucella infection; the findings are those of almost any bacteremia with pronounced intoxication." Infection now and then becomes localized to cause vegetative endocarditis, orchitis, osteitis, meningitis or subacute arthritis.

The chronic lymphogranulomatous form of brucellosis is characterized by enlargement and lymph nodes. Forbus presents an excellent detailed portrayal of the gross and microscopic appearance of lesions. He states: "The basic reaction is a progressive proliferation of the large monuclear cells of the reticuloendothelial system accompanied by the exudation of fibrin and sometimes by hemorrhage. This is followed by coagulative necrosis—and finally, by the proliferation of fibroblasts or the formation of a dense scar composed of reticulum."

Pathological findings in an Iowa case complicated by meningoencephalitis were reported by Hansmann and Schenken.[30] DeGowin and Borts[31] recently reported postmortem findings on a patient who developed vegetative endocarditis and a mycotic aneurysm of the femoral artery.

Symptomatology. The patient with clinical signs of brucellosis may have several or all of the following: fever, chills, sweating, weakness, malaise, headache, joint pains, backache, anorexia and loss of weight. These ten symptoms and signs are listed in the order of frequency of mention on 1,011 case reports completed by Iowa physicians.

Bierring,[32] in an analysis of 150 cases of brucellosis and based on his own experience with the disease, presents the following clinical picture:

"Emphasis should be placed on the character of the onset, the rigors, the chills with profuse sweating, the muscular and joint pains, the loss of weight, and the continued and persistent character of the fever curve.

"The usual onset is gradual and insidious in the development of noticeable weakness with accompanying tired feeling. The patient often seems quite fresh in the morning but by the latter part of the afternoon is so fatigued as to be hardly able to get about. A headache and backache of greater or lesser severity are often features of the onset. Likewise, loss of appetite, digestive distress and constipation are frequent early symp-

toms. After a few days, or possibly several weeks, the patient becomes conscious of a hot feeling mostly in the afternoon, and is usually surprised to learn that the temperature is above normal. A feeling of feverishness, and light rigors, and chills, are often first indications of fever. Again, the onset may be ushered in abruptly by a severe chill and rapid rise of temperature, followed by very profuse sweating, and this, with the general muscular pains, gives the impression of a profound infection."

FOLLOW-UP OF PATIENTS ILL IN 1943 AND 1942

In May, 1946, a letter and follow-up form were forwarded to physicians of Iowa to secure information regarding the duration of illness and present condition of patients whose blood serum in 1943, (several had onset of illness in 1942) showed positive agglutination in dilutions ranging from 1:40 to 1:2560, as notified from the State Hygienic Laboratory of the Iowa State Department of Health.

1. *Duration of Illness.* Among 114 patients for whom replies were received from more than 100 attending physicians, four deaths occurred in which brucellosis was the primary or contributory cause (3.5 per cent mortality). Duration of illness in fifty-four (47 per cent) was within three months; in sixty-eight (60 per cent), within five months and in eighty-seven (76 per cent) within one year. Eight patients (7 per cent), had symptoms lasting more than a year, twelve (10.5 per cent) were ill over two years and one patient longer than four years. Six reports did not indicate the duration of illness.

2. *State of Health in May,* 1946. In the series of 114 patients under consideration, sixty-five or 57 per cent were stated as being "well as ever"; the health of thirty others (26 per cent) was "fair." Not all of the reports were complete, some of the patients having changed location (including several who served in World War II), rendering follow-up unsatisfactory.

Considering separately fifteen of the twenty patients in this series whose illness lasted a year or longer, seven of these were stated as being "well as ever in May 1946," the condition of seven was "fair," while the health of but one was "poor." Current complaints of eight of these patients who are known to have had acute brucellosis three or four years ago and some of whom may now be regarded with a degree of certainty as showing residual effects of the disease (chronic brucellosis) include the following: weakness, inability to do a day's work, occasional fever of moderate grade, night sweats, pains in muscles.

DIAGNOSIS AND LABORATORY AIDS

A diagnosis of brucellosis based entirely on clinical manifestations cannot be made with accuracy; certain laboratory procedures are essential to confirm the clinical findings.

1. *Isolation of Brucella.* The recovery of brucella from the blood of a patient whose symptoms suggest brucellosis, establishes the diagnosis beyond a doubt. The isolation of brucella not only confirms the clinical diagnosis, but also makes possible identification of the species of organism through resort to Huddleson's dye-method of differentiation, and thereby aids greatly in the tracing of infection to its source in the hog, cow, sheep or goat.

There are physicians who would limit the diagnosis of clinical brucellosis to patients who show a positive blood culture. Although it is likely that bacteremia is present during the early febrile period in the majority of all acute brucellosis cases, brucella is actually isolated from a relatively small percentage due to factors such as the following: (1) failure to secure blood cultures during the early febrile stage of the disease; (2) failure to use media adapted

for growth of brucella; (3) lack of facilities for incubating cultures under CO_2 and (4) discarding of cultures after a few days of incubation. Insistence on positive blood culture as the sole criterion to confirm clinical diagnosis causes many cases of brucellosis to be missed or overlooked.

2. *The Agglutination Test.* This laboratory procedure is next in importance to the blood culture as a confirmatory diagnostic aid. Brucellosis reports as notified officially in most if not all of the forty-eight states are, so far as known, based primarily on positive agglutination findings to confirm the physician's clinical diagnosis.

Both rapid and slow methods of agglutination are trustworthy. When negative at first, agglutination tests should be repeated at weekly or ten day intervals. Although a negative test does not exclude the disease, agglutinins in diagnostic dilution (1:40, 1:80 or higher) are apt to be present at one time or another in as high as 90 per cent of all cases showing early clinical manifestations.

The agglutination titer tends to decrease during the months following acute illness, and usually becomes entirely negative. On the other hand, brucella agglutinins in diagnostic dilution are known to persist for several years in the serum of some patients, even as long as ten years after apparent clinical recovery.

3. *The Skin Test.* A positive skin test with brucellergen or other brucella antigen connotes an allergic response which, like a positive Mantoux or tuberculin reaction, probably means that exposure to infection has occurred either recently or at some time in the past. A positive intradermal test in the absence of positive blood cultures or positive agglutination reactions does not warrant the conclusion that the symptoms of which the patient complains are due to brucellosis. Surveys in Iowa have revealed positive skin tests in from 10 to 25 per cent of apparently healthy persons. The authors agree with Goodman[33] that clinical brucellosis is ordinarily of sporadic occurrence and that reliance on a positive skin test to confirm the clinical impression leads very often to uncertainty if not to error in diagnosis.

4. *The Opsono-Cytophagic Test.* Experience of various workers with the opsono-cytophagic test and interpretation of results have been summarized by Huddleson.[10] There is need for further study to determine the value of this method as a laboratory aid in clinical diagnosis.

5. *Need for Additional Diagnostic Aids.* According to Alice Evans,[34] the most reliable indicator of infection, apart from the recovery of brucella, is a positive agglutination reaction in dilution of 1:40 or higher. Miss Evans states that "there is great need for further perfection of methods for the diagnosis of chronic brucellosis."

TREATMENT—PROPHYLAXIS

An Iowa physician epitomized his own experience with treatment of brucellosis by stating that while one patient tended to improve rapidly under any method of care, another failed to respond no matter what therapeutic measures were applied.

Early diagnosis of the acute case and confinement of the patient to bed or complete rest until ten days to two weeks after the temperature has become normal are of paramount importance. Rest, preferably in bed, should be supplemented by encouragement of the patient, a high caloric diet, forcing of fluids, control of constipation and other symptomatic care as may be required. Faithful adherence to the complete rest regimen is apt to be rewarded by decrease in duration of illness, also by lessened likelihood of complication, recurrence of symptoms and chronicity.

Simpson[35] and Harris[36] give detailed consideration to the management of this disease.

Some patients react favorably to use of brucellin or to non-specific protein therapy. Penicillin and sulfatherapy in combination, are frequently though not always effective; the former may prove life-saving should the patient's lowered resistance render him vulnerable to secondary infection (e.g., streptococcal pharyngitis). An Iowa physician, meticulous in the keeping of hospital records and in securing repeated blood cultures as a means of appraising therapy, has noted excellent results clinically in three patients following the use of streptomycin.

When illness from brucellosis is prolonged, this may be due to localization of infection. Such localization is now and then amenable to treatment. An Iowa boy, aged nine, had symptoms lasting over two years, including repeated attacks of tonsillitis; remarkably prompt recovery of strength was noted following tonsillectomy. Another patient improved rapidly after removal of an ovarian cyst, fluid content of which yielded a pure culture of Brucella suis. A third patient developed osteomyelitis of the second and third lumbar vertebrae, which apparently healed under orthopedic care. Strongly positive agglutination reactions led to diagnosis of brucellosis in these cases.

Decrease in the prevalence of brucellosis of man depends upon collaboration with official agencies and the veterinary medical profession in measures designed to drain the reservoir of infection in animals. Such measures include: (1) discovery and control of sources of infection in hogs and cows, sheep and goats; (2) reducing to a minimum the hazard of direct contact with animals; (3) improved sanitation on farms and in the packing industry; (4) active immunization of animals (calfhood vaccination) and (5) careful supervision and pasteurization of all dairy products.

There is urgent need for evaluation of a vaccine similar to that used by Kolmer and associates,[37] for active immunization of individuals whose occupation entails daily exposure to brucella infection.

COMMENT

Infection caused by brucella organisms is always more widespread in a community than is indicated by the occurrence of active clinical cases. Information as to the extent of infection is obtainable through agglutination and skin test surveys carried out among groups of individuals subject to exposure through direct contact with brucellosis in animals or through use of unpasteurized milk from infected dairy cows. Results of recent surveys as here presented confirm those of a previous report.[12] Survey findings indicate that the ratio of latent or subclinical to clinical cases of brucellosis may be 8:1 or higher. The incidence of latent infection, as demonstrated by positive agglutination reactions and also by positive blood cultures, appears to be an important factor to explain or account for the usually sporadic occurrence of clinical cases.

The late Wade Hampton Frost[31] was the first to demonstrate clearly the inter-relationship between infection, immunity and disease in the epidemiology of diphtheria and poliomyelitis. It seems evident that the principles enunciated by Frost also hold true for brucellosis.

The practice of diagnosing brucellosis, acute or chronic, on the basis of no more than a positive skin reaction is (1) often inaccurate, (2) tends to bring the matter of diagnosis of the disease into disrepute among physicians who rely on positive blood cultures and positive agglutination findings and (3) multiplies by many times the actual occurrence of clinical brucellosis.

SUMMARY AND CONCLUSIONS

1. Information is presented pertaining to epidemiologic, clinical and laboratory

aspects of brucellosis of man as caused by the three species of brucella.

2. In addition to the porcine and bovine varieties, the caprine strain (Br. melitensis) is now known to be endemic in Iowa; thus far hogs (not sheep or goats) have proved to be the source of infection and Br. melitensis has been isolated from swine tissues.

3. Agglutination and skin test surveys help to reveal the extent of brucella infection in families or groups and the role played by latent infection in association with clinical cases of the disease.

4. Recovery of brucella and positive agglutination reactions in diagnostic dilution (1:80 and above) are two laboratory mainstays to confirm the clinical diagnosis.

5. A positive intradermal test should under no circumstances be used as a sole basis to confirm the clinical impression of acute or chronic brucellosis.

6. Prevention of human illness from this disease is dependent upon control and eradication of the disease in animals which serve as the source of infection; upon reduction to a minimum of direct contact with animals and their tissues and upon thorough pasteurization of all dairy products.

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Seminars on Rheumatic Fever

The Relationship of Streptococcal Infections to Rheumatic Fever*

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IN order to evaluate the evidence which indicates a close relationship between rheumatic fever and infections with hemolytic streptococci, it is advisable to have a clear picture of our current knowledge of these microorganisms, and also of certain immunological reactions in which they take part.

The streptococci comprise a large class of bacteria which grow as round or oval forms in chains of various lengths. In addition to pathogenic varieties, there are many which are not pathogens, some of which play a useful industrial role; for example, in the ripening of certain cheeses. Others may serve as test objects for the detection of biological or chemical products. In the earlier years of bacteriology, classification techniques were based largely upon the ability of the respective strains to grow in or on various artificial media or to split certain chemical agents; and today some of these characteristics are still valuable aids in studying some streptococci.

Another method of classification consisted in applying the name derived from the pathological condition from which they were isolated; i.e., Streptococcus pyogenes from purulent conditions, or Streptococcus erysipelatis from erysipelas. It is now clear that either purulent or erysipelatous conditions can be induced by the same strain of streptococci as well as by strains belonging to different immunological groups or types; hence this system of classification has little validity.

Still another, and relatively useful appellation, derives from the action of streptococci on red blood cells. If, when grown in or on media containing intact erythrocytes, the hemoglobin is changed to methemoglobin and the majority of the blood cells remain intact, the designation green or viridans is employed; if the hemoglobin is released from the red cells and not changed to methemoglobin, the streptococci are termed hemolytic; if the red cells and their contents are not visibly affected, the streptococci are designated as indifferent. The three Greek letters alpha, beta and gamma have also been used to describe these three classes of streptococci.[1]

The most modern, and in many respects the most useful system of classification, is based on immunological procedures which doubtless stem from the chemical characteristics of various streptococcal components. This immunological classification was developed by Dr. Griffith[2] in England, but more completely by Dr. R. C. Lancefield[3] in this country.

Streptococci are divided immunologically first into groups, and then the members of

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For description of methods of grouping and typing, see appendix at the end of this lecture, page 182.

certain groups are further subdivided into types.

Group Specificity. In 1924, Hitchcock[4] found that hemolytic streptococci, isolated from a wide variety of human diseases, possessed in common a serologically active carbohydrate which was designated as C substance. This fraction C was found to react specifically with the serum of animals immunized with any strain of hemolytic streptococci subsequently shown to belong to so-called group A, and failed to react with serum of animals immunized with a series of strains isolated from lower animals. Subsequently Lancefield[5] showed that another group-specific carbohydrate substance was present in streptococci isolated from many cases of bovine mastitis; and thus a new group, B, was discovered. Eventually, several other groups were recognized, the most characteristic sources of which are set out in Table I.

TABLE I
MOST CHARACTERISTIC SOURCE OF VARIOUS GROUPS OF STREPTOCOCCI (LANCEFIELD)

GROUP	MOST CHARACTERISTIC SOURCE
A	Man
B	Bovine mastitis
C	Most streptococcal animal diseases
D	Cheese; enterococci and other human saprophytes
E	Normal milk
F	"Minute" — Man: nasopharynx, usually nonpathogenic
G	"Minute" and large — Man: nasopharynx, usually nonpathogenic
H	Man: nasopharynx, usually nonpathogenic
K	Man: nasopharynx, usually nonpathogenic
L	Dog
M	Dog

While the primary sources shown in Table I represent the environment in which the respective members of the several groups usually occur, they may also find conditions suitable for their growth in other environments where they may even be pathogenic. These usual and unusual distributions of streptococcal groups are shown in Table II.

These facts are of more than mere academic interest. While members of group A are responsible for most streptococcal diseases in man, members of several other groups have been isolated from various parts of the human body, where occasionally they possess disease-inducing capacities.

TABLE II
PATHOGENICITY OF SEROLOGIC GROUPS OF HEMOLYTIC STREPTOCOCCI

Animal species	Streptococcal groups: Chief pathogens	Usually saprophytes, occasionally pathogens	Saprophytes (apparently)
Man	A	B, C, D, F, G, H	K, L
Monkey		A, G	C
Cattle	B, C	A, G	D, E, H, L
Horse	C		
Dog	G, L, M		C
Chicken	C	A (?)	
Swine	C	E, L	G
Goat	C	E, L	G
Sheep	C	E, L	G
Fox	C	M	
Ferret	C	M	
Rabbit	C	A, B	
Guinea pig	C	A, B	
Mouse	C	A, B, C	

Again, the milk coming from cows having mastitis due to streptococci belonging to groups B or C can be used for human consumption with relative impunity. When, on the other hand, as occasionally happens, a cow develops mastitis due to group A streptococci, her milk is a grave menace; and the distribution of such contaminated milk has lead to many epidemics of septic sore throat. Incidentally, such epidemics are often followed shortly by epidemics of rheumatic fever.[6] In contrast, it should be noted that human infections with streptococci belonging to groups other than A have not been shown to induce rheumatic fever.

An experimental predicament that arises from the normal distribution of the various groups among the several animal species is the difficulty of infecting an animal artificially with groups to which that species is

not usually susceptible, at least not to the extent encountered when the microorganisms operate in their "normal habitat." This makes analogies derived from animal experiments difficult of interpretation when applied to human maladies.

While the carbohydrate C fraction is useful in grouping streptococci, other biological phenomena depending upon its existence are not known. There is no group-specific agglutination; and the amount of group-specific C antibodies present in a given serum does not bear any relationship to such non-type-specific agglutinating capacity as that serum may possess. While the C substance is fairly constant in amount in a given strain, variations in the amounts of this substance seems to have no direct bearing on the virulence of the respective microorganism. This C carbohydrate forms part of the streptococcal cell from which it can be separated by complete disintegration of that cell. When so separated, it is non-toxic for animals.

Types among Group A Streptococci. The strains of streptococci comprising group A are further divisible into immunological types on the basis of their content of two different type-specific components, the so-called M and T substances.[7]

1. *Type-Specific Protein M.* This antigen is found in the variants of group A streptococci which form mucoid or matt colonies on solid media.[8] It apparently is mainly situated near the surface of the streptococcal cell. It is readily destroyed by certain proteolytic enzymes and by strong alkalies, but resists the action of fairly strong HCl. This property renders it extractable from the cell with HCl, and allows of further partial purification. Such extracts form type-specific precipitates when mixed with the properly absorbed sera of rabbits which have been hyperimmunized with the homologous strains; and such reactions are utilized in the precipitin typing technics.[9,10] By applying this technic, over forty different types have been identified and there are probably many others still not identified.

Certain strains of a given type will also agglutinate type specifically with homologous immune sera; and on this basis Griffith[11] developed a slide agglutination technic for identifying several types; but this technic sometimes leads to confusing results due to the presence in the streptococcal cell of other immunologically reactive components.

2. *Type-Specific T Substance.* A second type-specific component, the T substance, was also discovered by Lancefield.[12] It is destroyed by strong acids, but resists the proteolytic action of such enzymes as trypsin or pepsin. It is a strong agglutinogen. While certain strains have M and T substances both belonging to the same type, among strains belonging to certain other types there are T agglutinogens which are common to several types. Two such series have been described:[13] One comprising types 4, 24, 26, 28, 29 and 46 have closely related T substances but type-specific M antigens; and a second series comprising types 15, 17, 19, 23, 30 and 47 contain another common immunologically distinct T substance. These T substances shared in common by several types make it impossible to distinguish by agglutination tests a member of one of these series from another belonging to the same series. One other peculiarity among certain types has been described: Types 10 and 12[14] possess common M antigens but immunologically distinct T agglutinogens.

In animals artificially infected, type-specificity with respect to immunological protection runs parallel to the specificity as determined by M anti-M precipitin reactions, and not by T anti-T agglutination unless the M and T antigens of a given strain belong to the same type. The passive protecting capacity of a given type-immune serum also closely parallels its anti-M con-

tent; but no similar protective relationship has been demonstrated with respect to the T antigens and their corresponding antibodies in a given serum. Furthermore, humans or animals[15] infected with group A streptococci of a given type are resistant for fairly long periods to reinfection with that type, but are readily infected with strains of heterologous types. This fact has important epidemiological connotations.

3. *P Substance.* Other substances which can be extracted from the bodies of streptococcal cells are the P antigens. They probably comprise a mixture of nucleoproteins, which have neither group nor type specificity. In fact, similar substances are extractable from other cocci and bacilli. It is difficult to remove some of them completely from extracts containing M and T antigens; and this fact makes it somewhat hazardous to interpret immunological tests, both *in vivo* and *in vitro*, when reagents are employed containing mixtures of type-specific components, M or T, and non-specific nucleoproteins.

The four classes of substances above discussed are all apparently a part of the group A streptococcal cell. The three components C, T and P are usually fairly constant in any given strain, although occasionally either the C or T antigens may disappear from a strain which retains all of its other antigenic components.[16] The M antigenic content of a given strain is, on the other hand, quite variable. Highly virulent variants produce large amounts of M, lowly virulent variants little or none. By serial animal passage a strain of low virulence and with poor M-producing capacity can often be made highly virulent with a correspondingly large M content. Strains isolated from the nasopharynges of patients who have carried them for a long time, so-called "carrier strains," usually elaborate only small amounts of M antigen or even none. Such strains often grow in "glossy" colonies on blood agar, and must be identified immunologically by means of anti-T agglutination reactions because their low content of M makes it impossible to extract enough for a precipitin test, while their more stabile T content makes them agglutinable. Such glossy carrier strains have little virulence, and probably little invasive capacity.[17]

The only type-specific antibody which it has so far been possible to demonstrate *in vitro* in human sera, which is not complicated by cross reactions, is the so-called bacteriostatic antibody.[18] The presence of this antibody is shown by the phagocytosis of virulent M-producing strains of streptococci. This phagycytosis by normal leukocytes is effectuated by three components: complement; a thermostabile factor; and an antibody which is type specific with respect to M antigens and not to T antigens. Application of bacteriostatic tests has demonstrated the development of type-specific antibodies in the blood of patients following group A streptococcal infections,[18,19] and as might be expected, in that of patients with rheumatic fever. The difficulties repeatedly encountered in searching for type-specific antibodies with agglutination, precipitin, and complement-fixation technics probably stem in large part from the great difficulty in preparing antigens used in these technics in a pure form, free from other immunochemical components which give cross reactions with the various antibodies which inevitably occur in the sera of animals or humans infected with microorganisms containing a variety of such components.

Extracellular Streptococcal Products. In addition to the substances contained within the bacterial cell, group A hemolytic streptococci elaborate soluble substances into the media in which they have been grown; and antibodies reacting with some of these substances have been described. The following soluble products have been fairly well studied:

1. *Streptolysins* (*hemolysins*). The reagents responsible for hemolysis of erythrocytes are elaborated into the broth or blood agar. Todd[20,21] has described two: (a) Streptolysin O, which is oxygen labile, and while produced chiefly by most hemolytic members of group A, it is also produced by some strains of other groups; (b) streptolysin S, which is oxygen stabile and which seems to be peculiar to group A streptococci. Animals immunized with the soluble streptolysin O produce an antibody which combines with this lysin and thus inhibits its hemolytic activity. Similarly, antistreptolysin O appears both in the blood of animals and in that of about 90 per cent of human patients following group A streptococcal infections. In man, the amount of this antibody formed is very roughly proportional to the intensity and duration of the streptococcal infection. Todd[22] has further shown that certain strains of group A streptococci do not elaborate streptolysin O, but that their hemolytic capacity is due to other streptolysins. Persons infected with such streptococci naturally would not develop any antistreptolysin O; and it seems reasonable to conclude that at times the impossibility of demonstrating this antibody in the serum of patients is due to infections induced by these peculiar strains. It has been repeatedly shown that about 90 per cent of patients suffering from acute rheumatic fever have pathological amounts of antistreptolysin O in their serum. Such findings are strong evidence for predicting a close relationship between rheumatic fever and streptococcal infections. It should be emphasized, however, that the abnormal antistreptolysin O titre is not pathognomonic of rheumatic fever but of the precursory streptococcal infection.

Todd, Coburn, and Hill[23] have reported that during attacks of rheumatic fever patients have a lower average content of antistreptolysin S in their sera than occurs in streptococcal infections without rheumatic fever sequelae. Weld[24] has described a very potent poison with hemolytic and other cytotoxic properties which is extractable from streptococcal cells. It apparently is the same as streptolysin S.[25]

2. *Fibrinolysin.* Many members of group A and some of groups C and G elaborate into broth in which they have grown a substance which appears to dissolve fibrin.[26] Christensen[27] has recently demonstrated that the lytic system consists of a zymogen normally present in human serum, and which remains inactive until it is combined with an activator, fibrinolysin*, which is produced by the streptococci. In the serum of many patients suffering from streptococcal infections there develops an antibody, antifibrinolysin, which apparently neutralizes this activator, and thus prevents it from combining with the fibrinolytic proenzyme. The observation that most patients with rheumatic fever develop relatively large amounts of this antifibrinolysin in their serum, which normally contains little or none, is additional evidence that they suffer from the effects of a streptococcal infection.

3. *Erythrogenic Toxin.* This toxin, which is formed in broth by many group A streptococcal strains, by an occasional group C strain, and apparently by a very rare Staphylococcus aureus, appears responsible for the rash of scarlet fever.[28,29] It is neutralized by antierythrogenic antibody which appears in the blood of scarlet fever patients with recovery. Similar neutralizing antibodies are demonstrable in the sera of many persons with no history of scarlet fever, but who have probably suffered previously from mild or subclinical infections with streptococci having weak erythrogenic-toxin forming capacity.

It is now generally conceded that the difference in the clinical picture between

*This has been designated as streptokinase.

two persons infected with an erythrogenic-toxin producing strain, one of whom develops scarlet fever and the other a simple nasopharyngitis, is that the second had in his serum antibodies against the erythrogenic toxin while the first did not. Because the rash-free patient usually has fever, malaise, and other general signs of intoxication, it appears logical to conclude that the streptococci elaborate other toxins, the nature of which is not well defined.

From the standpoint of inducing rheumatic fever, streptococcal infections, with or without a rash, have similar significance. Postscarlatinal rheumatism has been long recognized and its relationship to rheumatic fever has been the subject of many polemics.[30] The advantage of using scarlet fever as an example of precursory streptococcal infection lies in its clear-cut clinical peculiarities, and in the fact that it has long been a reportable disease, from which fairly reliable statistical analyses may be reconstructed.

4. *Hyaluronic Acid.* This substance is present in the capsular substance of streptococci, especially members of groups A and C, whence it readily diffuses into the surrounding broth. Some observers suggest that it is responsible for the virulence of group A streptococci,[31] and that in this respect it resembles the capsules of pneumococci. There is, however, quite convincing evidence that virulence of these streptococci is more closely related to their type-specific protein M content.[32,33] Hyaluronic acid is one of the principal components of the umbilical cord, synovial fluid, and the ground substance of connective tissue. As might be expected from its widespread distribution in the animal body, antibodies against hyaluronic acid have not been demonstrated. Whether hyaluronic acid, or a compound of which it is a component, has any pathogenic significance with respect to rheumatic fever is still an open question.

CASE REPORTS

In order to set forth the evidence of the close relationship between group A hemolytic streptococcal infections and rheumatic fever, case reports of four patients are presented. These illustrate various clinical courses in which there were different manifestations indicating the probable, possible, or questionable existence of rheumatic fever. On the charts are shown the evidence of streptococcal infections and the antibody responses to those infections. In all instances the streptococcal infection was scarlet fever; but we have seen many instances where simple tonsillitis or streptococcal nasopharyngitis played a similar role.

CASE I. This patient had a typical scarlet fever with type 19 streptococci in the nose and throat. Following a course of sulfadiazine, this strain apparently disappeared and was replaced by type 6, which in turn was replaced by a group A streptococcus of undetermined type, which the patient carried in his nose and throat for many weeks. Finally, this type disappeared, and eventually type 19 streptococci again appeared and were present in small numbers until the twentieth week. The signs of scarlet fever disappeared about the tenth day; and there was a quiescent period (phase II) for about two weeks, followed by a typical attack of rheumatic fever in which most of the clinical symptoms and signs of the disease were present. The electrocardiogram revealed partial heart block; and a few days later an apical systolic murmur appeared which persisted. This indicated the presence of mitral valvulitis. As the patient did not tolerate salicylates well, his antirheumatic therapy consisted of aminopyrine (pyramidon) which controlled the symptoms; but the laboratory evidence of persisting infection was of several months duration.

CASE II. This patient had a similar but less severe attack of scarlet fever due to type 19 streptococci. In this case the first course of sulfadiazine did not clear up the carrier state with respect to streptococci. A second course given from the thirty-seventh to the forty-

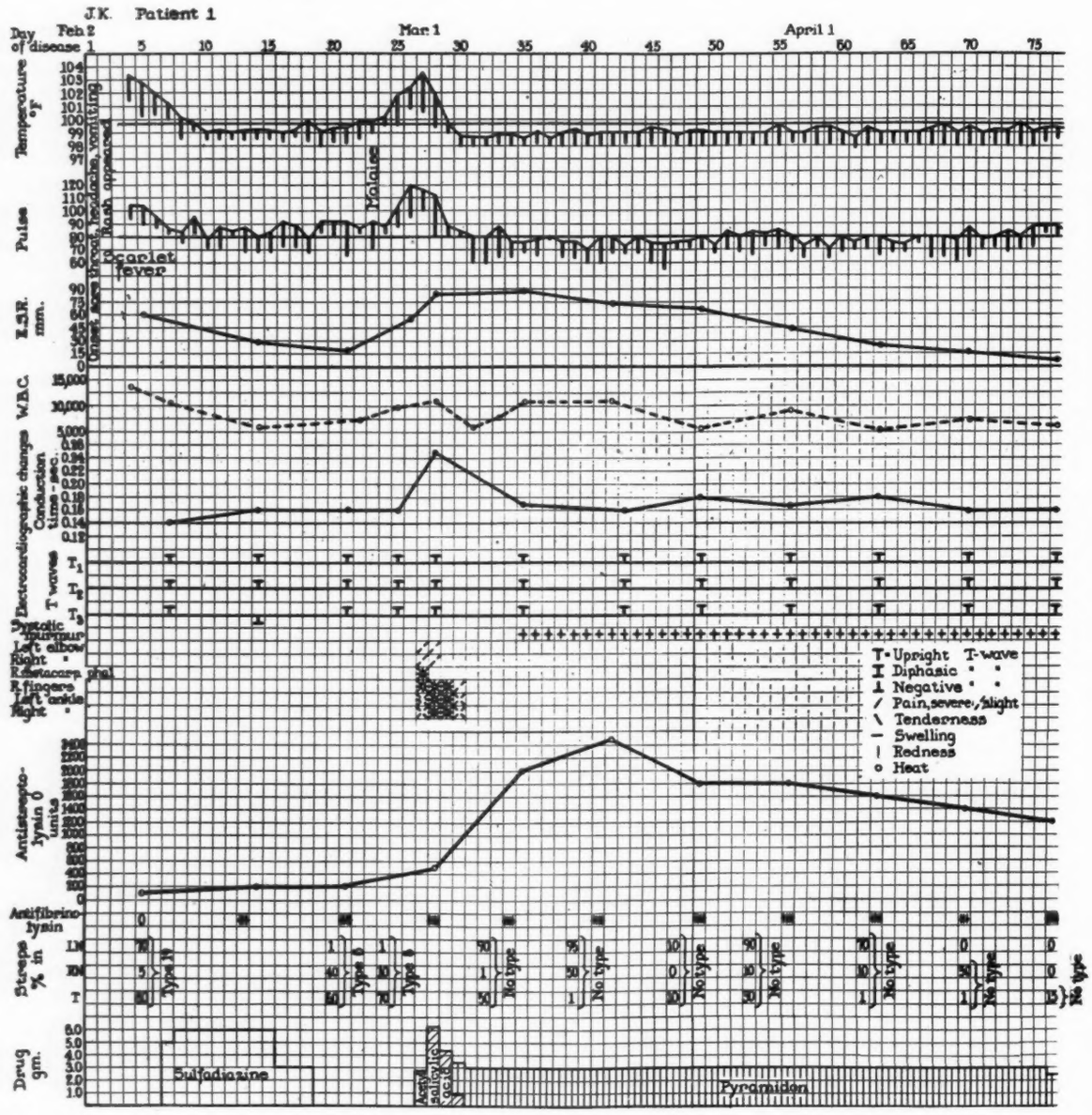

FIG. 1. Streptococcal infection (scarlet fever) followed by a two weeks' quiescent phase, then a typical attack of rheumatic fever with both polyarthritis and carditis.*

second day, however, caused permanent disappearance of these microorganisms from the nose and throat. After a quiescent period (phase II) of over four weeks, there was evidence of active carditis as indicated by dropped beats, markedly prolonged conduction time, and the appearance of a mitral systolic murmur which persisted thereafter. Noteworthy was the absence of fever and the very slight evidence of arthritis, which consisted merely of pain in the spine for three days. A reversion of the ESR from normal during phase II to 15 mm., and of leukocytosis was additional evidence indicating a rheumatic attack.

CASE III. In this patient the scarlet fever was more marked and prolonged than in the previous patient. Type 19 streptococci were evidently the offending microorganisms. These persisted for over eight weeks in large numbers in the nose and throat except for a period when the patient was under the influence of sulfadiazine. Here again, after a quiescent period

* These studies were carried out in collaboration with Dr. R. F. Watson and Dr. Sidney Rothbard.

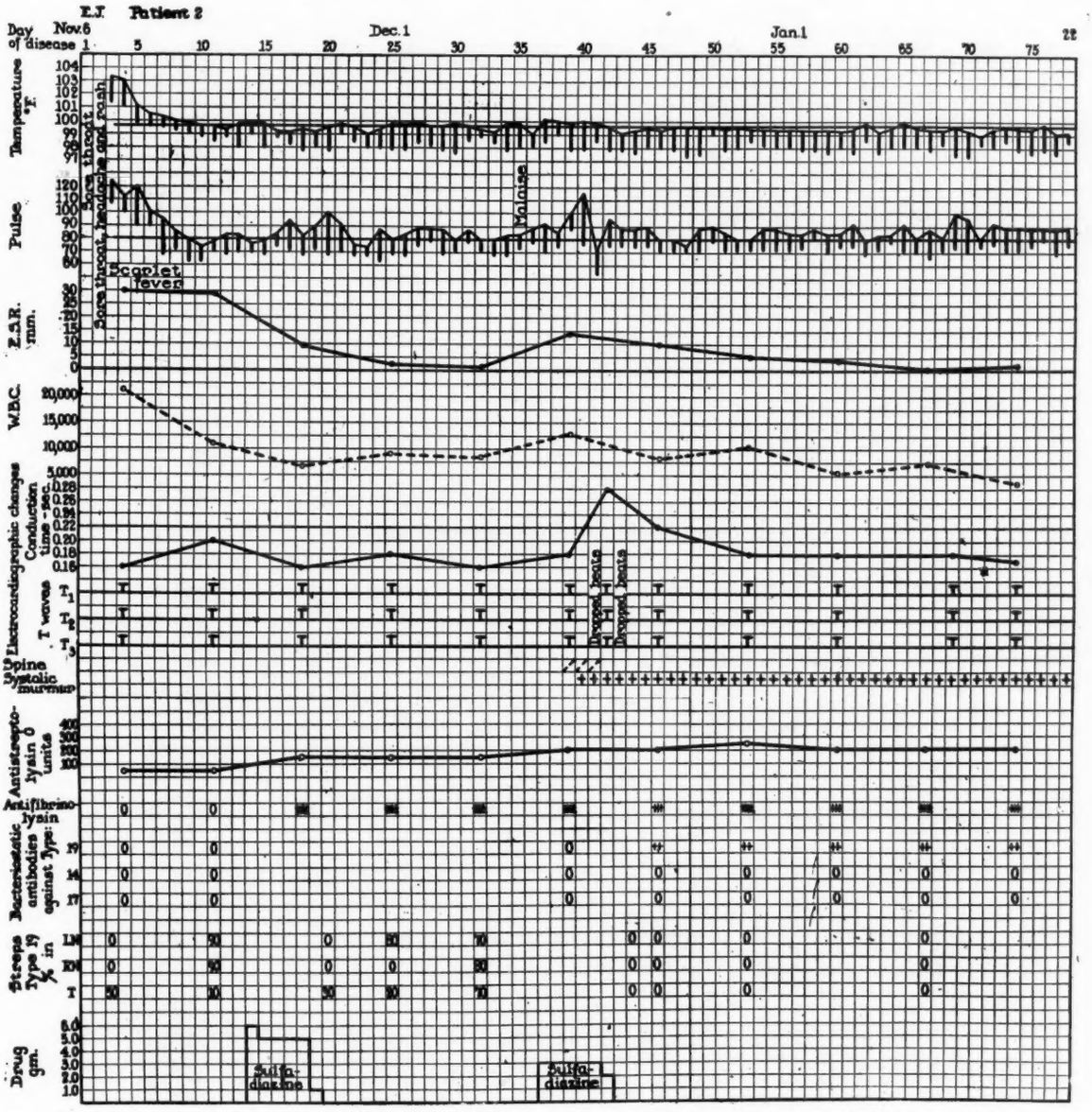

FIG. 2. Streptococcal infection, followed by a four weeks' quiescent period, then definite carditis, but no fever and minimal arthritis.

(phase II) of four weeks, there was evidence of a rheumatic-like attack, which consisted of a recurring abnormal ESR, leukocytosis, and a prolonged conduction time in the EKG. Obvious signs of rheumatic fever were conspicuous for their absence; hence one must hold in abeyance the certain diagnosis of this disease.

CASE IV. In this fourth case, the findings indicated a possible, but very questionable, rheumatic attack occurring much earlier, and consisting of pain in both elbows and slight precordial distress lasting four days. This was followed shortly by abnormal electrocardiographic evidence such as diphasic T_1, then during the third week negative T_1 and T_2, which disappeared gradually by passing through a diphasic stage. Later T_3 became negative for about three weeks. The only other evidence of possible rheumatic fever was a temporary recurrence of abnormal ESR at the beginning of the sixth week. In contrast to the first three cases, it is to be noted that the evidence of cardiac and arthritic involvement occurred early during what would normally be designated as phase I. It is very questionable, therefore, whether these should be designated as truly

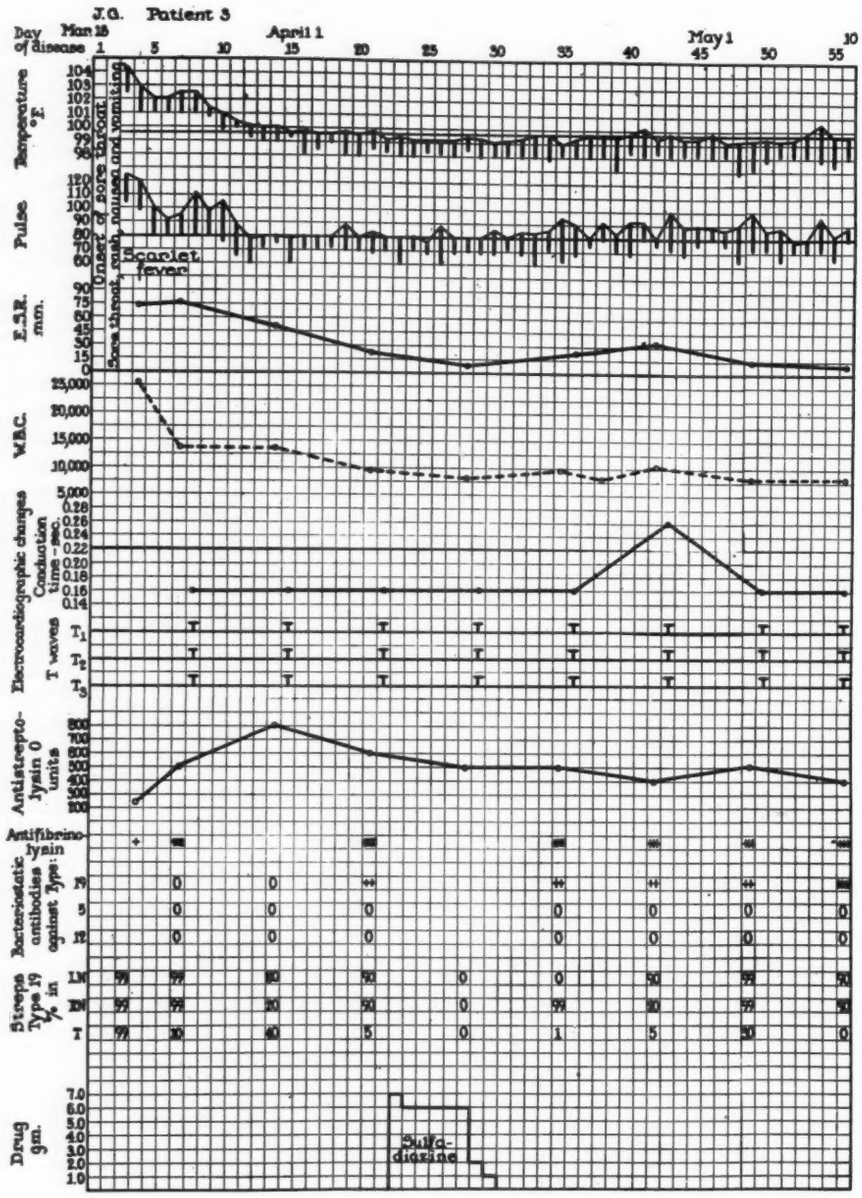

FIG. 3. Streptococcal infection, four weeks' quiescent period, then temporary indication of carditis.

rheumatic, because many patients have pains in the joints during the acute streptococcal episode; and electrocardiographic abnormalities similar to those shown by this patient are not infrequent in many febrile conditions.

It is to be noted in this case that the administration of sulfadiazine was followed by a permanent disappearance of streptococci from the nose and throat. This, in our experience, is an exceptional occurrence, and it must not be forgotten that a certain portion of patients with streptococcal nasopharyngitis show similar disappearance of the microorganisms from their respiratory passages without the assistance of antibiotics.

The time of appearance and the intensity of streptococcal antibodies such as antistreptolysin O, antifibrinolysin, and bacteriostatic antibodies in the various patients are indicated on the charts. In all there was a fairly rapid appearance of antifibrinolysin. In all the antistreptolysin O titre became distinctly abnormal; but there seems to be no

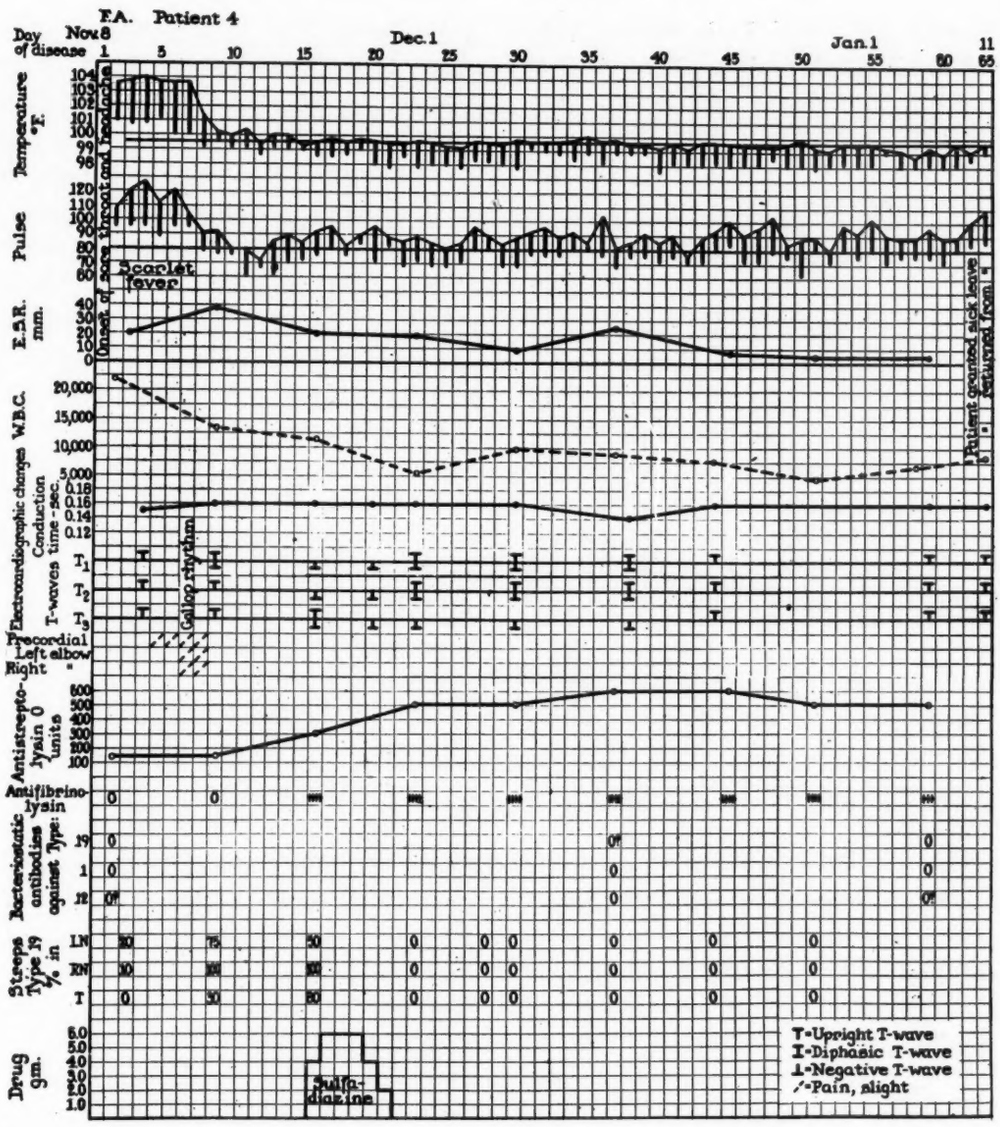

FIG. 4. Severe streptococcal infection accompanied by arthralgia and followed immediately by electrocardiographic signs of cardiac involvement.

general rule as to when this may occur. In two, no definite bacteriostatic antibodies were demonstrated; while in the other two, they appeared in moderate concentrations fairly early, but were not present in high concentrations until very late.

These four cases bring up the question of the difficulties in making a diagnosis of rheumatic fever. In Case I the clinical picture during the acute febrile episodes, phases I and III, were so typical of their respective states, and the development of a mitral systolic murmur subsequent to the migratory polyarthritis was so characteristic, that the diagnosis was easily made without resort to any laboratory aids. In Case II the difficulties increase. There was an initiatory streptococcal infection, viz., scarlet fever; and during a relatively long phase II, all symptoms and clinical and laboratory signs of infection disappeared, except that type 19 streptococci persisted in the nasopharynx. Such a patient would ordinarily have passed from observation, for he probably would have disregarded the malaise on the thirty-fifth day. He remained

afebrile: and the slight pain in the spine from the thirty-ninth to the forty-first days might easily have been overlooked. The dropped pulse beats might have been considered as due to extra systoles, but their true nature was revealed in the electrocardiograms which showed a partial heart block, a fairly frequent occurrence in active rheumatic carditis. The concurrent appearance of a mitral systolic murmur which persisted thereafter was fairly conclusive evidence of valvulitis. Recurring abnormal ESR and distinct leukocytosis all fit into the pattern of an acute rheumatic episode. In fact, except for the differences in fever, severe intoxication and migratory polyarthritis during phase III, cases 1 and 2 are not dissimilar.

Case III, on the other hand, presented increasing diagnostic difficulties for during phase III there were only slightly increased heart rate, slight leukocytosis and moderately elevated ESR, but a distinctly, though temporarily, elevated conduction time, as revealed in the EKG. There was no stethescopic evidence of carditis while he was under observation. According to current diagnostic criteria, it would be difficult to have the diagnosis of rheumatic fever accepted. How then is he to be regarded? Probably if he contracts subsequent group A streptococcal infections, his liability to develop a definite rheumatic attack will not be that of normal persons, i.e., about 10 per cent, but that of previously rheumatic patients, i.e., from 25 to 50 per cent.

Among 110 young adults with scarlet fever observed in The Rockefeller Institute Hospital,[34] seven showed during phase III evidence of a possible but questionable low-grade rheumatic episode, similar to that illustrated in Case III; four resembled Case II, but three showed no evidence of valvulitis; and eight had distinct rheumatic fever.

Many similar reports,[30] and also comparable findings following streptococcal nasopharyngitis,[35,36,37,38] suggest that in allowing most patients to escape from observation during the two months following their acute streptococcal infection, we are failing to detect many early cases of low-grade rheumatic fever. It should be more widely recognized that many patients have rheumatic fever with little or no polyarthritis. Probably the incidence of the disease has not decreased to the extent indicated in hospital reports. Pathologists still see many cases where exitus is attributable to rheumatic carditis or chronic cardiac valvular disease; and the cardiac clinics continue to have a case load of from 30 to 40 per cent of rheumatic cardiacs among their total enrollment.

Additional evidence of the common occurrence of arthritis-free rheumatic fever occurs in the findings of Levy and his coworkers.[39] Among approximately 5,000 draftees who were rejected from induction for some form of heart disease, about one-half had definite signs of chronic rheumatic valvular disease. Two-thirds of this group denied any history of rheumatic fever or chorea; in fact, rheumatic disease was diagnosed for the first time when they appeared for the draft. It is quite possible that these men, while not having had true rheumatic polyarthritis, had a syndrome comparable to that described above; namely, a streptococcal infection followed by mild rheumatic disease without typical manifestations.

Further evidence of the close relationship between rheumatic fever and group A streptococcal infections rests in the demonstration that prevention of such infections by small, long continued doses of the sulfonamides prevents recurrence of rheumatic fever in rheumatic subjects exposed to such infections, while control patients not receiving these drugs develop the usual proportion of rheumatic recurrences following streptococcal infections.[40,41,42] Similarly, in

large streptococcal epidemics in military installations, where the epidemics were controlled by sulfa prophylaxis, there was a corresponding diminution in new cases of rheumatic fever.[43,44] A valid objection might be advanced: The sulfonamides might have had a prophylactic effect on the development of an unrecognized hypothetical "rheumatic fever virus." When, however, sulfa-resistant strains of streptococci appeared, and hence the epidemics were no longer amenable to sulfa prophylaxis, rheumatic fever occurred the same as among untreated controls.

It is furthermore noteworthy that neither the sulfonamides nor penicillin therapy have any beneficial influence on the manifestations of rheumatic fever, once they have appeared. Even the administration of large doses of these drugs during phase II, that is after the precursory streptococcal infection is established, apparently does not prevent the appearance of the rheumatic phase. These observations provide additional support to the probability that neither the sulfonamides nor penicillin has any effect on a hypothetical "rheumatic fever virus," and add weight to the conclusion that their beneficial influence upon rheumatic fever is in the prevention of the precursory streptococcal infection.

In weighing the evidence concerning the possible etiological role of streptococci in rheumatic fever, it should be emphasized that other infections have never been implicated as inducers of this disease. Respiratory diseases due to pneumococci, gram-negative bacteria, influenza viruses, or hypothetical viruses causing the common cold, have never been shown to set up a sequence such as is found between group A streptococcal infections and rheumatic fever. Furthermore, atypical pneumonia which frequently is accompanied by evidence of infection with the nonhemolytic streptococcus MG,[45] does not act as a precursor to rheumatic fever. This points to the advisability of stopping loose talk about the connection between "respiratory infection" and rheumatic disease, and of emphasizing the unique sequential relationship between hemolytic streptococcal infections and rheumatic fever until the existence of this phenomenon is firmly established in the minds of physicians, public health workers and the laity. Once this peculiar relationship is understood, then it is comprehensible how rheumatic subjects can mingle with people having colds or influenza without the threat of rheumatic recurrences unless these diseases are complicated by group A hemolytic streptococcal infections.

If the highly probable and unique role of group A streptococcal infections in inducing rheumatic fever were generally accepted, then several important movements would logically eventuate. Instead of devoting the major portion of our efforts to the care of patients with chronic heart disease, much as these patients deserve attention, we would vigorously study more effective means of preventing the streptococcal infections whence flow the rheumatic fever sequelae. We would alter our attitude towards the apparently benign nature of scarlet fever, tonsillitis, and streptococcal pharyngitis because so rarely are they immediately fatal. During the years that usually lapse between the initiating streptococcal infection, the subsequent rheumatic attack, often asymptomatic, and the final picture of cardic failure, so many events intervene, so many physicians attend the patient, that significant relationships often become obscured.

How group A streptococcal infections induce rheumatic fever has not been established. Much remains to be learned about the various chemical and enzymatic components which are present in the streptococcal cells or are liberated into their environment during their growth. More

must be known about host-parasite relationships with respect to these components. Our ignorance, however, should not become obstructive; it should not cause us to neglect the clearly established relationships so repeatedly mentioned in this communication and so often emphasized by many competent observers.[46] To quibble over the question of the etiology of rheumatic fever and to permit that quibbling to prevent applying effectively the partial, but very practical knowledge of this subject we now possess, is to serve our patients less satisfactorily than is within our power, and the public less effectively than it deserves.

DISCUSSION

DR. TARAN: Thank you Dr. Swift, for the scientific discourse on our present knowledge of the hemolytic streptococcus and the relationship of the streptococcal infections to rheumatic disease. This dissertation opens so many questions that we may easily drift off the subject of discussion. I suggest, therefore, that we try as far as possible to stick to the question of the hemolytic streptococcus and its relationship to rheumatic disease.

QUESTION 1: Has it been established that, when certain strains become resistant to the sulfonamides they belong to a specific type of hemolytic streptococcus?

DR. SWIFT: No. Sulfa-resistant strains have been discovered among several types, i.e., 1, 3, 6, 17, 19 and 30. These types have recently caused numerous epidemics in which the exposed populations have received mass sulfadiazine prophylaxis.

QUESTION 2: Is it good policy to let a scarlet fever patient out of bed after the symptoms have subsided?

DR. SWIFT: That depends upon the symptoms shown by the patient and upon both the clinical and laboratory signs of continued active infection. Rather than following a rule that covers all cases, it is probably more important to make erythrocyte sedimentation rate determinations every week or ten days for at least two months and to concentrate further observations on those patients with continuously high rates and on those in whom the ESR increases to abnormal heights after an intervening return to normal.

QUESTION 3: From the clinician's standpoint, are there any tests that one can do on a patient with scarlet fever which would justify us in letting the patient up?

DR. SWIFT: One is justified in allowing a scarlet fever patient up when the temperature and pulse are normal, there is no leukocytosis, and the ESR is fairly normal. This would probably be good practice with patients following streptococcal nasopharyngitis.

QUESTION 4: Am I justified in concluding from your remarks that if a patient is discovered to have a streptococcal infection, and is given adequate sulfonamide therapy or penicillin, that we might prevent an onset of rheumatic disease?

DR. SWIFT: You are not. This question has been covered in the body of this lecture.

QUESTION 5: Would you say that scarlet fever or another streptococcal infection is part of the rheumatic syndrome rather than that rheumatic disease is a result of the streptococcal infection?

DR. SWIFT: The first concept hardly seems logical because most streptococcal infections are not followed by rheumatic fever. On the other hand, most if not all cases of rheumatic fever are preceded by streptococcal infections; and it would, therefore, be more logical to say that the rheumatic syndrome is part of a phase of streptococcal infections.

QUESTION 6: Why should we not consider any type of streptococcal sore throat as belonging to the same category as scarlet fever, with respect to rheumatic fever?

DR. SWIFT: We probably should.

QUESTION 7: It has been said that rheumatic fever occurs commonly in families. Do you think that if we could give sulfanilamide to every member of the family where one case of rheumatic fever is known to exist we might prevent other members from coming down with rheumatic fever?

DR. SWIFT: If this prophylactic treatment were continued long enough and at proper times, it would probably be effectively prophylactic with respect to rheumatic fever with the provision that the patient was not exposed to sulfa-resistant streptococci.

QUESTION 8: How often does the streptococcus, which is found in the first phase, disappear in the second or third phase?

DR. SWIFT: Probably in approximately 25 to 35 per cent. This disappearance of streptococci in the interval between phase I and the onset of the rheumatic attack has been one of the stumbling blocks preventing certain observers from accepting the etiologic rôle of streptococcal infections in rheumatic fever. The argument is as follows: If the streptococci are the etiologic agents, they should be present and demonstrable at the time the active rheumatic manifestations occur. One should not lose sight of the fact, however, that this disappearance of the streptococci from the nose and throat does not prove that they are not still lurking and active in more inaccessible structures such as the paranasal sinuses or the lymph nodes draining these areas.

QUESTION 9: Is it true that the organisms that were present in phase one increase once again in the third phase?

DR. SWIFT: This is not true as a rule; for example, as mentioned in the previous answer, in about a quarter to a third of the patients the streptococci disappear. Usually in the other two-thirds there is a tendency towards a decrease in the number of recoverable streptococci, although in some there is a constant discharge of large numbers. These are probably the most dangerous persons with respect to distributing streptococci among healthy people. Doubtless, one of the most important health measures that might be devised would be to change the carrier streptococcal state of these patients.

QUESTION 10: Is it true that the incidence of rheumatic disease is greater at certain times of the year than at others? Is it also true that certain localities have a low incidence? Do these seasons and these localities also have a low incidence of streptococcal infections?

DR. SWIFT: The incidence of rheumatic fever shows a seasonal curve analogous to that of group A streptococcal infections. Also, the incidence of rheumatic disease follows closely the geographic distribution of streptococcal infections. These relationships are not absolutely parallel for in the tropics certain areas seem to have a comparatively small number of streptococcal infections, with a still smaller proportion of rheumatic manifestations. It seems as though these climatic conditions might favor the lack of development of rheumatic fever.

QUESTION 11: Have any cases of rheumatic fever been described which did not have a streptococcal infection preceding the onset of rheumatic fever? Let us say, in two hundred cases of rheumatic fever, how many are not known to have had phase I and phase II?

DR. SWIFT: One cannot give an exact answer to this question because there are only two ways of determining the presence of phase I: (1) by clinical observation which may be misleading because streptococcal infections of very low grade may occur without setting up observable clinical respiratory infections; and (2) bacteriologically and immunologically. If we could study all of these cases bacteriologically and immunologically for the presence of antibodies against streptococcal components, practically all would be shown to have a

phase 1 prior to the attack of rheumatic fever.

QUESTION 12: In your studies, what percentage of patients could be prevented from having rheumatic fever by preventing streptococcal infections?

DR. SWIFT: We cannot answer this question from our work; but all studies on this subject indicate that the large majority of patients could be prevented from developing rheumatic recurrences if they receive continually small doses of the sulfonamides before the attacks of rheumatic fever; again with the provision that they were not infected with sulfa-resistant strains.

QUESTION 13: With our present knowledge of the relationship of the streptococcus to rheumatic disease, have we the right to say that this relationship is the beginning and the end of the story?

DR. SWIFT: No; as I have attempted to point out in the concluding part of this lecture.

QUESTION 14: Or have we simply the right to say that the streptococcus may be one of the factors?

DR. SWIFT: It is probably one of a number. It is the only one about which we have fairly definite information. There is of course another question, that of the soil in which the streptococcus acts; in other words, the precursory attuning of the human tissues which will determine the course of the streptococcal infection. Dr. May Wilson will probably present much evidence suggesting that hereditary factors may condition the tissues. Other possibilities are subject to experimental demonstrations which doubtless will be made before the final answers are available.

APPENDIX

TECHNIC OF GROUPING AND TYPING STREPTOCOCCI

The following description of the technic for grouping and typing streptococci in capillary pipettes is taken largely from the original article by Swift, Wilson and Lancefield[10] but slightly modified in the light of subsequent experience, especially in the use of pointed capillary pipettes for group testing.[47]

Apparatus. Capillary pipettes for *typing* are made from stock capillary tubing* 1.0 ± 0.2 mm. in external diameter which is broken into 7.5 cm. lengths. The external surface is cleaned with soft paper tissue but the inner surface is not cleaned because chemical treatment interferes with capillary action. The pipettes are placed in suitable glass test tubes.

Capillary pipettes for *grouping* are made from tubing 1.5 ± 0.2 mm. in external diameter. This is broken into 12 to 13 cm. lengths; the middle of each length is heated in a narrow flame (such as is made by a fish tail burner or the pilot of a Bunsen burner) and when melted, drawn to a very fine point by quickly pulling on the two ends of the tubing. Thus two conically pointed pipettes are formed with fine openings about the diameter of a hair. The outer surface of these pipettes is cleaned with paper tissue, and they are placed in test tubes containing at their bottoms absorbent cotton on which the conical points rest. If strong grouping sera are available, 1.5 mm. pipettes may be prepared in simple 7.5 cm. lengths like the 1.0 mm. tubing. The test tubes containing the pipettes are capped with *unsized* paper, then sterilized by dry heat or by autoclaving, followed by drying in an incubator.

Serum Containers. Containers for sera in current use consist of two parts. The first is

* The capillary tubing is made by the Kimble Glass Company, Vineland, New Jersey, and can be obtained from laboratory supply houses. It is described by that company as No. 46485 capillary pipette tubing, made of neutraglas (N-51A glass), not individually gauged, and varies in outside diameter from 0.7 to 1.0 mm. with 0.2 mm. wall. There are approximately 500 thirty-four inch lengths to the pound. For group classification, larger tubes with outside diameter of 1.2 to 1.5 mm. are used; 1 pound contains approximately 300 thirty-four inch lengths.

a small screw cap vial 45 mm. long and 15 mm. in diameter, with a neck having an inside diameter of 9 mm. The second is an inner container made of glass tubing with an external diameter of 7 to 8 mm. and an internal diameter of 5 mm. From this tubing a goblet-shaped cup is made, about 20 mm. deep, and standing on a solid stem and foot. The stem is cut so that the combined height of the stem and cup is 42 mm. The inner container which holds about 0.2 cc. is placed in the vial, and the cap attached. (Fig. 5.)* Each vial, with the cap loosened, is wrapped in paper and sterilized in the autoclave. The vials are filled from the stock serum bottles with aseptic precautions. The sediment is allowed to collect in the bottom of the cups; and when loading the capillary pipettes care must be taken not to stir it up or to draw it into the pipettes.

Vial Holder. A wooden block holds two rows of vials, one behind and about an inch above the other. The holes to receive the vials should be $\frac{5}{8}$ inch in diameter and $\frac{3}{4}$ inch deep and $\frac{1}{4}$ inch from margin to margin. A small piece of plasticine in the bottom holds the vials firmly so that they do not turn when the caps are unscrewed. (Fig. 6.)

Capillary Pipette Stand. This consists of a wooden block 10 in. long, $1\frac{1}{4}$ inches wide and $\frac{7}{8}$ of an inch deep. A groove $\frac{1}{4}$ inch wide and deep is cut lengthwise on one side, and is filled with plasticine. Labels are written on narrow strips of ruled paper and fastened in front of the groove. One stand is used for each extract when the complete set of sera is employed. (Fig. 7.)

Reading Equipment. A dull black screen is placed beneath and a few inches behind an electric light so that the tubes may be observed with back-lighting against a black background. A fluorescent lamp is superior to an ordinary incandescent bulb. The tubes are examined with a hand lens of about 5 diameters magnification.

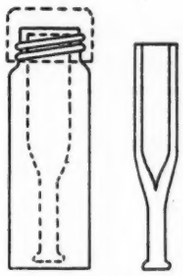

FIG. 5. Serum container in screw cap vial; detail to show shape.

Reagents.

(1) N/5 HCl
(2) Buffered N/5 NaOH made as follows:

Na_2HPO_4 (anhydrous salt)	5.786 Gm.
KH_2PO_4 (anhydrous salt)	3.532 Gm.
dissolved in N/5 NaOH	1000.00 cc.

(3) N/20 NaOH
(4) N/20 HCl
(5) 0.1 per cent solution of thymol blue
(6) 0.1 per cent solution of phenol red

All solutions should be kept in pyrex containers, and any solutions containing precipitates should be discarded. Extracts prepared with buffered NaOH, when mixed with the precipitating sera, must not produce a degree of cloudiness that would interfere with the readings. If this occurs repeatedly, a simple N/5 NaOH solution may be substituted for the buffered alkali.

Media. Todd-Hewitt Broth[48] *(Modified)—Beef Meat Infusion Base:* Cut away as much fat as possible from fresh beef heart or horse meat. Chop or grind the lean meat fine and to each pound add 1,050 cc. of distilled water. Stir, and with a sieve skim off the small particles of fat which arise to the surface. Place the meat and water mixture in the ice box overnight. The next morning heat to 85°C. and maintain this

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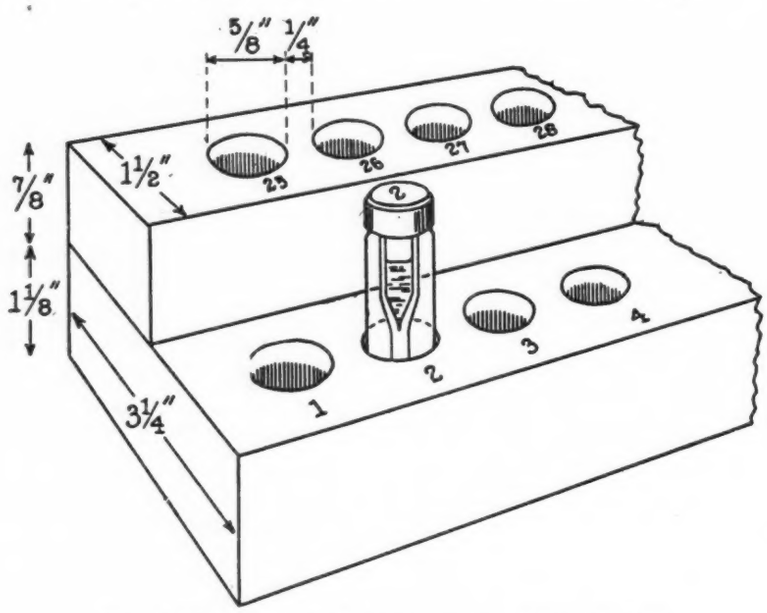

FIG. 6. Vial holder with one serum container in place.

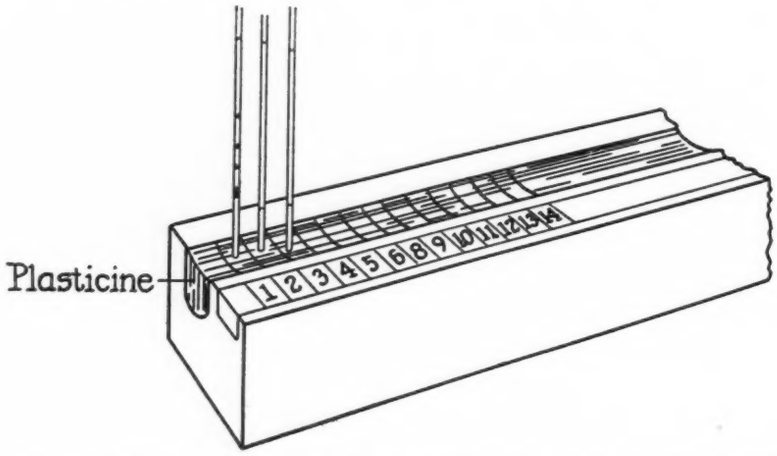

FIG. 7. Capillary pipette stand with three pipettes in place; precipitate in first tube indicates positive reaction with type 1 serum.

temperature for one-half hour. Filter the broth through coarse filter paper.

To each liter of the above infusion add 20.0 Gm. of neopeptone. Adjust pH to 8.0 with N/1 NaOH and add the following:

NaCl	2.0 Gm.
$NaHCO_3$	2.0 Gm.
Na_2HPO_4, anhydrous	0.4 Gm.
Glucose	2.0 Gm.

Boil for fifteen minutes and filter through filter paper. Tube in 40 cc. amounts* and sterilize in the Arnold for one hour on three successive days. The final pH should be 7.8. The fifteen minutes boiling should drive off the CO_2 prior to Arnolding. If not, a precipitate may be formed during sterilization. If this happens, the broth must be filtered and the pH readjusted under sterile conditions, followed by one hour in the Arnold. The anti-M proteinase[49] is inactive in this broth due to the use of neopeptone.

Blood broth for stock cultures may be prepared by adding two or three drops of defibrinated rabbit or sheep blood to 5 cc. of the Todd-Hewitt broth.

* For facilitating later manipulation, it is convenient to tube the broth in 50 cc. centrifuge tubes.

Good blood agar is needed for primary isolation of hemolytic streptococci from the throat and nose or from purulent material. It should be moist when inoculated and during incubation because dry media does not support growth well. Defibrinated rabbit or sheep blood is the most satisfactory blood. With the former, bacillus hemophilus hemolyticus shows hemolysis about the colonies, while with sheep blood this bacillus is inhibited.

Preparation of Bacterial Extract. Approximately 40 cc. quantities of Todd-Hewitt broth in 50 cc. centrifuge tubes are inoculated from the pure stock cultures and incubated at least eighteen hours or until a heavy growth is obtained. This is checked for purity of growth. The broth culture is centrifuged and the clear supernatant fluid is pipetted off or decanted.

The bacterial sediment is mixed with 0.4 cc. of N/5 HCl. A loopful of the suspension should give an orange red color with a drop of 0.01 per cent thymol blue, that is, the extractions should be carried out at a pH of 2.0 to 2.4. If necessary, more N/5 HCl is added to obtain this range.

The mixture is transferred to a pointed 15 cc. centrifuge tube and heated in a boiling water bath, shaken at three-minute intervals for ten minutes, cooled and centrifuged.

The clear supernatant fluid is decanted into a second centrifuge tube and a small drop of 0.01 per cent solution of phenol red is added, which colors the solution a distinct yellow.

0.3 to 0.33 cc. of double buffered N/5 NaOH is added drop by drop until a faint pink color appears. The first *faint pink* color is a pH of 7.0 and a good extract may have a pH between 7.0 and 7.8. If too alkaline, the extract is readjusted with N/20 HCl because non-specific precipitin reactions may occur when the pH of the extract is over 7.8. The slight precipitate formed during neutralization is discarded after centrifugation and the supernatant fluid which should be crystal clear is pipetted or decanted into small test tubes. This is now ready for testing with antisera, and is used both for grouping and typing.

Difficulties in grouping and in typing tests are generally traceable to faulty preparation of the extract. It is essential that extraction be carried out at a pH below 2.5. It is also important to keep the final volume small. Cloudy extracts may be caused by contamination, by the use of N/5 NaOH stored in non-pyrex glass containers, and by stirring up of the sediment in the bottom of the centrifuge tubes. Contaminated extracts may give false reactions. All glassware must be perfectly clean; and the acid and alkali solutions must be of accurate normality.

Preparation of Precipitating Sera. Several different grouping and typing sera are commercially available. The typing sera are prepared by immunizing rabbits with heat-killed vaccines of streptococci known to be rich in M antigens.[9] After the serum is thoroughly absorbed with a strain of heterologous type to remove non-type-specific precipitins, it must react strongly with homologous M extracts in order to be useful in this reaction.[12] The absorbed sera, preserved with merthiolate 1:10,000, are conveniently stored in small dropping bottles from which about 0.2 cc. are transferred by means of the droppers to the small serum containers. Care is taken not to draw the precipitate which sometimes accumulates in the bottom of these containers into the capillary pipettes while performing the tests.

Sterility. Sera must be handled aseptically and kept sterile at all times. Except when in actual use, they must be kept in the refrigerator. Contamination is a frequent cause of cloudy sera, and of both false-positive and negative tests.

Cloudiness. All sera, particularly when freshly prepared, develop a fine precipitate which settles to the bottom of the container. This does not indicate deterioration, and may be allowed to collect unless it makes the sera cloudy. Some sera retain slight opalescence; and the technician must be familiar with these in order to avoid confusion or false-positive tests. Cloudy sera may be cleared by centrifugation or filtration through small Seitz filters.

CAPILLARY PIPETTE GROUPING

Grouping may be performed either for final identification, or as a necessary step in sorting out members of group A. In the latter case, it is necessary only to differentiate group A streptococci from the other groups.

The capillary group A screening test is usually run with a number of extracts just before the performance of typings. The bacterial extract, serum vials, capillary pipette stands, 1.5 mm. conical pointed capillary pipettes, and the equipment for reading are arranged conveniently.

Procedure. The sterile pointed end of a 1.5 cm. capillary pipette is dipped into the proper grouping serum until a column of serum between 1.0 and 1.5 cm. long has been slowly drawn in by capillary action. This end of the pipette is then wiped with paper tissue and dipped into a drop of extract until an equal amount of extract has been drawn into the pipette. Air bubbles must not separate serum and extract. The pipette is again wiped, then the conical end is plunged 1 or 2 mm. into a lump of plasticine in order to seal the hair-sized opening. The lower open end of the pipette is then pressed against a roll of plasticine which has been previously placed on the strip of plasticine of a capillary pipette stand. Thus the pipette will be held in a vertical position without being plunged into the plasticine, and in this way the fluid in the top of the pipette will not be forced out by hydraulic pressure. The conical end of the capillary pipette should be inspected to see whether any extract has been forced out during this manoeuver, and if this occurs, the minute drop should be wiped off so that no film is deposited on the pipette.

If the procedure has been properly followed, the column of fluid will be at the upper part of the pipette, and the sealing of the hair-like opening will insure that it remains there. The surface of the portion of the pipette containing the fluid should not have been touched by the fingers, hence should be perfectly clean. The lower air-containing portion probably will be finger marked.

The technic outlined insures the least possible amount of mixing of the underlying serum and the overlying extract; hence there is a narrow zone in which the precipitate forms. When longer columns of serum and extract are drawn into the capillary pipettes, this interzone moves over a wider range, more mixing of the two reagents occurs, and the zone of precipitate is wider; hence the reaction may be less intense. The same difficulty exists when the reaction is set up in 1.5 mm. pipettes with both ends open as previously recommended; rapid mixing of the two reagents occurs; and the reactions may be so indeterminate that they often require confirmation in small test tubes.

Reading. Within five to ten minutes a positive reaction is shown by a cloudy white ring of very fine precipitate at the junction of serum and extract. With weak sera or extracts, a longer time may be required. If the pipettes are placed in the incubator at 37°C. for an hour, weak cross reactions with sera of other groups may occasionally occur; hence readings made within five to ten minutes probably indicate more specifically the group to which the streptococci under examination belong. Upon standing the precipitate formed early may redissolve, or

it may clump, and fall to the bottom of the column of serum.

Errors due to weak reactions may result from excessive mixing of the extract and serum. Another source of error is to let the test stand too long before reading. False readings may result from grease marks or other materials on the outer surface of the tubes over the zone of reaction. Extracts and sera should always be crystal clear before preparing the test, since hazy sera or sediment drawn into the capillary pipettes with the reagents may lead to false-positive readings.

The pointed 1.5 mm. capillary pipettes are satisfactory for most grouping tests, but in some cases, larger quantities of serum and extract are required.[9] Place ordinary medicine droppers, with the bulbs removed, in a plasticine block so that the small ends are completely sealed. Place 0.05 cc. of the respective sera into each. Slowly pipette about 0.05 cc. of extract into each tube allowing it to layer over the serum. Within thirty minutes examine for a white ring of precipitate at the junction of the extract and serum. If the reactions are not clear cut, make extract dilutions of 1:4, 1:8 and 1:16, and test these dilutions. Similar dilutions may be tested in the larger pointed capillary pipettes. If cross reactions are still encountered, make a new extract by the formamide method and retest. The formamide method is only applicable to preparing extracts for grouping.

Formamide Extract.[50] Centrifuge 10 cc. to 15 cc. broth culture until the bacteria are packed; remove the supernatant as completely as possible and discard; to the sediment add 0.2 cc. of formamide. Shake; and place the tube in an oil bath (automobile or mineral oil) at 150°–180°c. for fifteen minutes; cool; and add 0.5 cc. of acid alcohol (1 cc. concentrated HCl with 99 cc. of 95 per cent alcohol); and centrifuge. Transfer the *supernatant* to a clean centrifuge tube; add 1 cc. of acetone and centrifuge lightly; discard the supernatant. Add 2 cc. of normal saline to the *sediment;* shake, and add 1 drop of bromthymol blue indicator; then add sufficient 2 per cent sodium carbonate ($Na_2CO_3H_2O$, *not* "technical" sodium carbonate) to turn the extract blue. Centrifuge before use in the precipitin test.

CAPILLARY PIPETTE TYPING

In the presence of an epidemic due to hemolytic streptococci, both time and material may be saved by testing with the type serum covering the epidemic strain and with three to six other type sera as controls. Only if these give negative results need all of the sera be employed. Similar economies may be effected in testing repeated cultures from the same patient. These tests should be preceded by tests with group A serum.

Procedure. A container of capillary pipettes is mounted horizontally in plasticine; and capillary pipette stands, reading equipment, extracts and sera are arranged conveniently. Only those extracts are tested which have reacted with group A serum. The sterile end of a capillary pipette is placed in the serum until a column 1.5 to 2.0 cm. long has been drawn in by capillary action. It is next dipped into the extract, and an equal column is run in after the serum. If an air bubble separates the serum and extract, the pipette is discarded and another one set up. The column is allowed to run to the middle of the pipette, and the pipette is carefully wiped with soft paper tissue. It is then inserted into the plasticine of the pipette stand so that the serum is on the top of the extract. Similar preparations are made with each serum to be tested. (Fig. 7.)

Reading. As soon as a test has been set up, the tubes are examined for cloudiness with a hand lens and if foreign particles are present, the questionable pipettes are discarded and the test repeated. The pipettes

are incubated for two hours at 37°C. and preliminary reading is made. A final reading is made after the pipettes have stood overnight in the refrigerator. Most positive reactions will appear at the two-hour reading. When the extracts set up only against the common type sera give negative readings at this time, they may be set up immediately with the other sera. The following scale is used: ±, just visible; +, a few fine masses visible with the hand lens; ++, usually beaded throughout, visible with the naked eye; +++, and ++++, column filled with larger masses of precipitate. Each positive test must be interpreted in the light of the known reaction of the serum with homologous extract. One plus or weaker readings should not be accepted as diagnostic.

True cross reactions are very rare. False-positives are usually caused by improperly prepared extract, or cloudy contaminated sera or extracts, or by dirty tubes. Whenever a particular test is doubtful, it should be set up again with serum, saline and serum, and homologous extract controls. Confirmatory tests may be performed by using larger pipettes or small test tubes, or with dilutions of extract. A strain should not be considered as untypable until several tests have been made by different techniques.

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Heredity and Rheumatic Disease*

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ALTHOUGH most clinicians are allergic to numbers, the statistical approach to rheumatic fever which has engaged our attention for several years has provided a framework within which various aspects of rheumatic fever may be explored.

In a lucid consideration of general principles of epidemiologic procedure, Frost states the following:

"In collecting facts about the distribution of disease, the purpose in view is always to a better understanding of its nature, sources, means of spread and eventually of its control. This implies that the facts must be related to each other in such an orderly way as to establish a theory or philosophy of the disease, given sufficient scope and accuracy of observation, a conclusion as to the nature and spread of a disease may often be established quite firmly by circumstantial evidence well in advance of experimental observation. Moreover, many problems of disease transmission which are highly important from the standpoint of prevention are such that can be solved only by investigation of this kind. The weakness in conclusions drawn from circumstantial studies is usually chargeable not to basic defects in the methods of investigation but more often to paucity or inaccuracy of data, or to faults of logic in their interpretation."

This approach has already yielded valuable information as to the nature of rheumatic fever, particularly as it affects attacked families in a clinic population in New York City.

Genetic Risk. It was demonstrated in genetic and epidemiologic studies that hereditary factors were primarily responsible for the familial concentration of rheumatic fever. It was found that in these families the distribution of cases followed the general laws of recessive Mendelian inheritance.

Although at the present time the genetic susceptible child cannot be identified on the basis of recessive inheritance, the chance for each child in a family or group of families of known hereditary background to be susceptible can be expressed as follows: (Fig. 1.) If both parents are rheumatic, nearly every child will be susceptible. If one parent is rheumatic and the other parent is not rheumatic but a carrier, i.e., rheumatic fever is present among near relatives, each child has a 50 per cent chance to be susceptible. If neither parent is rheumatic but both parents are carriers, each child has a 25 per cent chance to be susceptible. If at least one child is rheumatic, it may be assumed that the parents are carriers. If one or both parents are non-rheumatic and non-carriers, susceptible children would be unlikely.

Genetic analysis of a series of rheumatic families revealed that the number of genetic susceptibles estimated in the families studied was found to be in close agreement with the final number of cases observed. (Fig. 2.) It may be postulated therefore, that distributed in the population, there are individuals who are susceptible or insusceptible to the development of rheumatic fever on a genetic basis. However, at the present time, it cannot be concluded that every genetically susceptible child will necessarily develop

* Seminar conducted at St. Francis Sanatorium for Cardiac Children, Roslyn, Long Island, on October 23, 1945.

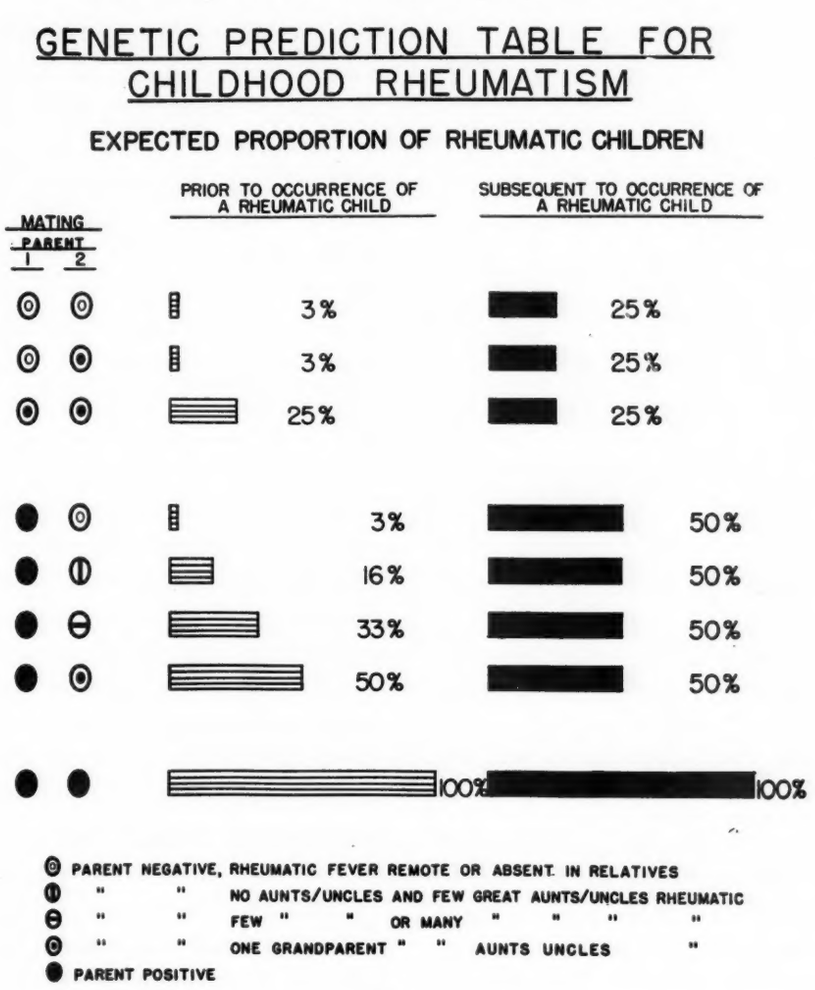

GENETIC PREDICTION TABLE FOR CHILDHOOD RHEUMATISM
EXPECTED PROPORTION OF RHEUMATIC CHILDREN
PRIOR TO OCCURRENCE OF A RHEUMATIC CHILD
SUBSEQUENT TO OCCURRENCE OF A RHEUMATIC CHILD
MATING PARENT 1 2
3%
25%
3%
25%
25%
25%
3%
50%
16%
50%
33%
50%
50%
50%
100%
100%
PARENT NEGATIVE, RHEUMATIC FEVER REMOTE OR ABSENT IN RELATIVES
" " NO AUNTS/UNCLES AND FEW GREAT AUNTS/UNCLES RHEUMATIC
" " FEW " " OR MANY " " " "
" " ONE GRANDPARENT " " AUNTS UNCLES "
PARENT POSITIVE

FIG. 1.

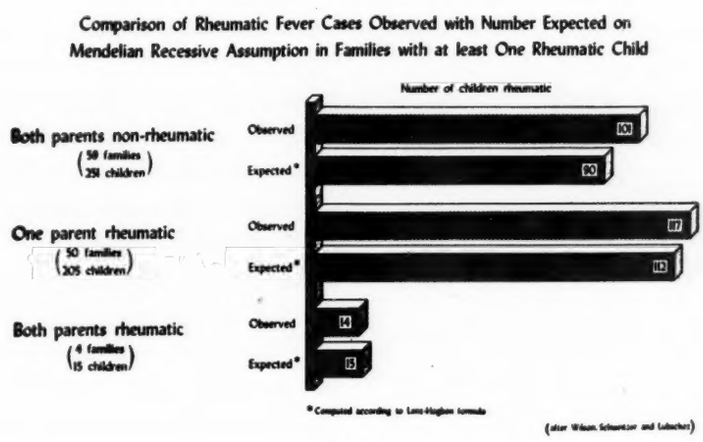

Comparison of Rheumatic Fever Cases Observed with Number Expected on Mendelian Recessive Assumption in Families with at least One Rheumatic Child
Number of children rheumatic
Both parents non-rheumatic
(50 families 251 children)
Observed
Expected*
100
90
One parent rheumatic
(50 families 205 children)
Observed
Expected*
87
112
Both parents rheumatic
(4 families 15 children)
Observed
Expected*
14
13
* Computed according to Lenz-Haghen formula
(after Wilson, Schweitzer and Lobscher)

FIG. 2.

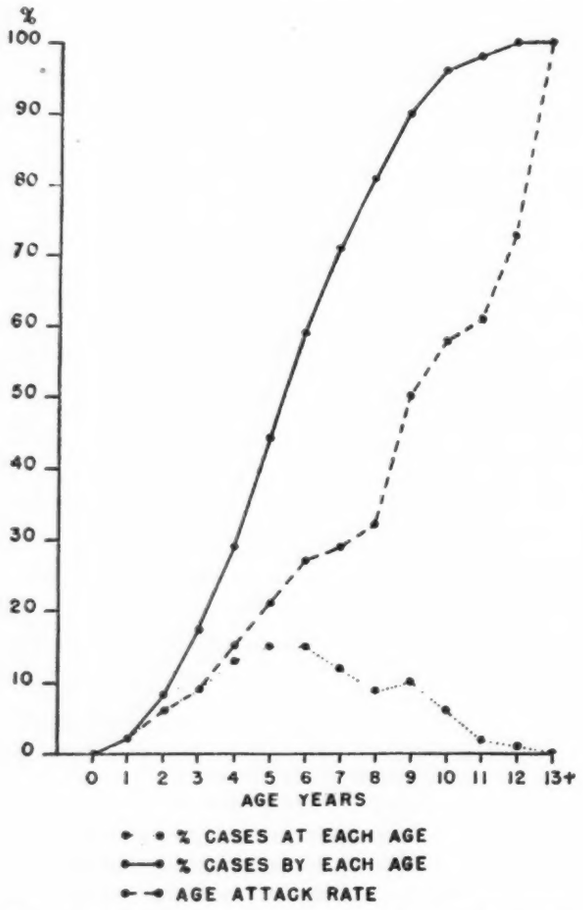


FIG. 3. Age factors derived from a rheumatic series of 688 cases of rheumatic fever at onsets.

rheumatic fever. It is probable that genetic analysis of a similar series of rheumatic families in comparable city populations would also show close agreement between the number of genetic susceptibles predicted and the number of cases observed. Whether the results of such genetic analysis made in Arizona or Louisiana or in a non-clinic population would be comparable must await the results of such investigations. It must be emphasized that the distribution of genetic susceptibles would not be expected to vary in various geographical localities and among diverse economic groups, although the frequency of rheumatic cases may. Should future studies of families reveal a difference in penetrance of the disease, methods for the control of rheumatic fever would be available. There is urgent need for such studies to be undertaken.

It is clear that there are certain persons distributed in the population whose genetic make-up predisposes them toward having rheumatic fever. This genetic predisposition is a constant factor, from birth to death. Whether the disease will develop is probably dependent upon other factors, both within the individual organism and in the environment.

Age Risk. The age expression of rheumatic fever is probably one of the most important factors in the evolution of the disease. It has long been observed that rheumatic fever usually develops during childhood, from the age of four years to puberty, with an average age of onset of about six years. As Dr. Paul has aptly stated, "the infant must grow up to be rheumatic." In other words, there is an age factor in rheumatic fever which must be taken into account as well as the genetic background. For example, an infant, both of whose parents are rheumatic and who therefore has almost a 100 per cent chance to be rheumatic on a genetic basis, would not be expected to show symptoms of the disease until he had reached the age of at least four years or more. If this child is brought to the clinic at the age of two years, and the parents wish to know whether he will develop rheumatic fever during the coming year, it would be wrong to state that there is a strong likelihood, because at the age of two, few potentially rheumatic children have the disease.

In order to make a statistical evaluation of the age risk in rheumatic fever, the incidence of case onsets at various ages was calculated. These incidence rates represent the average chance a genetically susceptible child has for developing rheumatic fever at any particular age. (Fig. 3.)

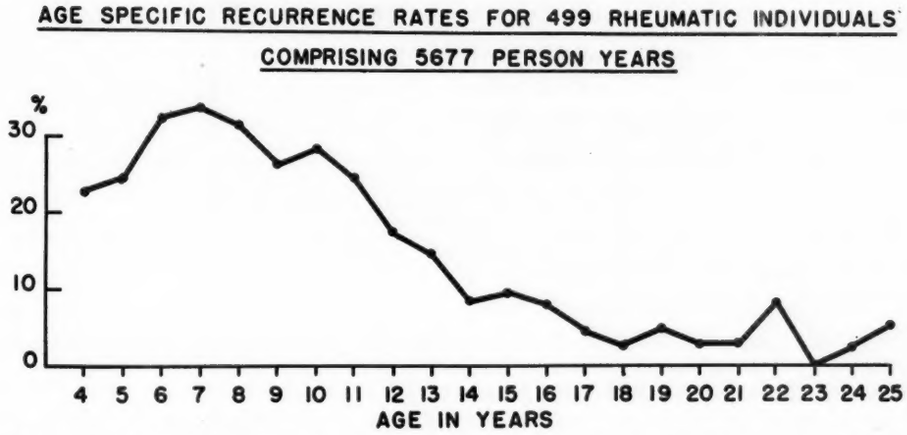


FIG. 4. Age specific recurrence rates for 499 rheumatic individuals comprising 5,677 person-years.

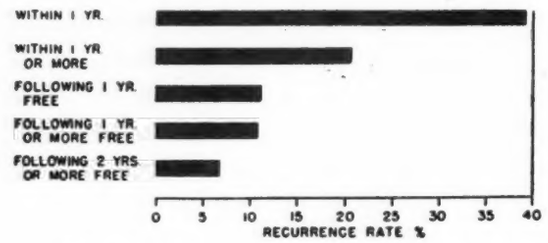
Genetic-age Risk. In 109 families studied, the genetic risk and the age risk were applied in combination, making it possible to predict the annual incidence of case onsets of primary and secondary cases, during the life experience of these families. That is, the intrafamilial pattern of spread of rheumatic fever was completely described by the use of age and genetic factors.

These observations are of epidemiological significance. They demonstrate that whatever factors are responsible for the onset of rheumatic fever among susceptibles, they were uniformly operative and effective during the entire life experience of these families. Furthermore, they demonstrate that rheumatic fever does not exhibit the usual characteristics of a communicable disease. It is unlikely that comparable observations could be obtained in any known infectious disease. On the other hand, similar findings might be demonstrable in a series of diabetic families. Should the genetic-age risk be found to be different in certain environmental situations, information about the rôle of specific environmental factors such as climate, diet and bacterial agents could be obtained.

It is not within the scope of this presentation to speculate as to the nature of the inherent defect or to interpret the age expression of the disease. It may, however, be concluded that heredity is primarily responsible for the familial incidence of rheumatic fever and that the age risk determines the time of occurrence of cases in the family.

It is to be emphasized that statements about heredity in any disease refer to explicit cellular and functional attributes and properties whose precursors have a concrete and real existence in the genes. The hereditary character may be responsible for abnormal physiologic, chemical or hormonal reactions in the genetic susceptible host. Frequently a variety of exogenous factors may be necessary for the expression of the condition in the susceptible host without which the conditions will fail to be expressed altogether. It is therefore necessary to evaluate the effect of non-genetic factors such as environment, diet and bacterial agents, on the aquisition of the disease in a susceptible host.

Risk of Recurrence. Awareness of the importance of rheumatic fever has stimulated renewed efforts for its prevention. Current etiologic concepts form the basis for prophylactic therapy. In the present state of our knowledge of rheumatic fever, this approach is valid. If prophylactic therapy proves suc-



The risk of recurrence in relation to the last rheumatic attack.

FIG. 5

cessful, in addition to the prevention of the disease, evidence for a basic etiologic concept would be obtained.

Here it is necessary to sound a note of warning if history is not to be repeated. It is necessary only to recall the premature reports of the prophylactic value of tonsillectomy.

The recently reported favorable results of sulfonamide prophylaxis have been widely accepted. Thomas, summating current published studies, observed that of 815 patient seasons over a period of seven years, incidence of recurrence was 1 per cent, compared with 10 to 35 per cent in untreated control groups. If these results are valid, the conclusion that all rheumatic children should receive therapy "day in and day out" would be justified.

However, critical analysis of published studies revealed that the individual studies did not meet the basic requirements for adequate biostatistical analysis. Selective and inadvertent bias characterized most studies. Rarely were alternate experimental and control patients selected. Frequently, patients were shifted back and forth from experimental to control groups. Such patients were usually uncooperative patients who refused treatment.

In many studies the experimental and control groups were not comparable because of differences in age constitution. In the majority of the studies, the groups were too small, and final conclusions were usually based on summated observations. This practice is only acceptable provided each study which is included represents a random selection of patients. In addition, diagnostic criteria and observation must be uniform, environmental conditions and age constitution comparable. The published studies which have been summated in rheumatic fever do not appear to meet these requirements. It is obvious that final judgment as to the validity of the etiologic concept and consequent preventive therapy which are based on these studies must be deferred.

The clinical course of rheumatic fever is characterized by frequent recurrence of manifestations of the disease and a varying number of intercurrent years of apparent freedom from symptoms. Since current etiologic concepts and consequent preventive therapy are based in large measure on a comparison of the number of recurrences among experimental and control groups of rheumatic patients, it seemed important to define the average risk for a recurrence of rheumatic fever.

Age-risk for Recurrence. To obtain a measure of the expected risk of overt recurrence of rheumatic fever (arthritis, chorea, active carditis), a series of 500 records of patients representing 5,600 person years of life experience under continuous medical supervision were selected for analysis. It was found that the average over-all risk for a major recurrence was 25 per cent for patients between the ages of four to thirteen years, 9 per cent for patients between the ages of fourteen and sixteen, and about 4 per cent for those seventeen to twenty-five years of age. It is obvious that the risk of recur-

rences varies significantly with the age of the patient. (Fig. 4.)

Of particular importance was the observation that the risk of recurrence during the year immediately following a major episode was twice as great as that following at least one year of freedom and three times as great as that following at least two years of freedom from symptoms. (Fig. 5.)

Contrary to expectation, the rate of recurrence was not found to vary in twelve consecutive calendar years or with the number or severity of previous attacks.

The expected risk for a major manifest recurrence of rheumatic fever which has been defined should prove useful in evaluating the results of prophylactic therapy. It is to be emphasized for future studies to avoid selective bias alternate experimental and control cases should be included for study. To prevent inadvertent bias, the age distribution of experimental and control groups should be comparable and the period between attacks for the two groups uniform. Such bias in a small series might be responsible for any differences observed. The biostatistical studies which have been described have provided fundamental data on the nature of the risk for developing rheumatic fever, for onsets as well as recurrences. They indicate that the most important factor in the pathogenesis of rheumatic fever is susceptibility of the host. Future studies may reveal the nature of the factors responsible for the development of rheumatic fever among genetic susceptibles of a susceptible age.

It has become increasingly clear that the risk for the development of rheumatic fever resides primarily in the host. Here is a fruitful field for future research.

DISCUSSION

DR TARAN: The subject of heredity and rheumatic disease is now open for discussion. Are there any questions?

QUESTION: Is there any way of predicting how many members in a family will have rheumatic disease when both parents are known to stem from rheumatic families?

DR. WILSON: Yes. Numerical factors have been carefully worked out by geneticists for recessive inheritance in many abnormal conditions, i.e., albinism. It is a relatively simple analysis. For prediction, a tabulation of the number of families of one or more children according to parental status is made. Multiplying by the genetic factor on families of each size the number of susceptibles expected may be obtained.

QUESTION: The story is told, that a mathematician once set out to prove the law of probability by tossing dice to see what the chances were of getting seven or eleven. He found that the law of probability did not operate in this experiment. The number of sevens and elevens was significantly different from the mathematical prediction. However, to his great amazement, it was found that when the dice were weighed carefully, one of the dice weighed a fraction of a milligram more than the other. In other words, one of the dice was weighted only slightly, and it was this error that upset the mathematical law. Was inadvertent bias excluded in your studies?

DR. WILSON: Yes. Various tests indicated that there was no selective bias in the material analyzed.

QUESTION: Are there any factors in the individual or in his family history which makes him particularly susceptible to repeated attacks? Can we say that an individual that gets repeated attacks is one who has a higher degree of rheumatic disease in his family tree than the one who has only a single attack?

DR. WILSON: I do not know. We have obtained no evidence to indicate different degrees of inherited susceptibility.

QUESTION: Can we really say that the incidence of recurrences of rheumatic fever

declines with the age of the patient? Or do the manifestations in adult life evade diagnosis? We all are familiar with the fact that a patient may give a history of one acute rheumatic attack in childhood but will show consistent and progressive cardiac damage as years go by. Many of us have seen patients who have developed minimal cardiac damage in childhood and have apparently had no recurrent attacks throughout adult life, but at forty years of age are found to have markedly enlarged hearts with multiple valvular damage.

DR. WILSON: I do not think that is the complete story. While it is true that new murmurs will be heard that were not heard before, in the absence of recognizable recurrent rheumatic attacks, the findings may represent damage sustained during the original active process. This may take years to develop. We are all familiar with the fact that acute rheumatic fever is not limited to the childhood years and that many adults have acute attacks. But I believe we do not have sufficient evidence to say that so-called subacute rheumatic disease is present in all adult rheumatics who appear to develop progressive cardiac damage.

QUESTION: Isn't it true that rheumatic heart disease and rheumatic fever have been transmitted by injecting blood of rheumatics into normal individuals?

DR. WILSON: No. Rheumatic fever as such has never been transmitted. You are undoubtedly referring to the work that was done in England recently. What happened in that experiment was that blood from people who were suffering from an acute rheumatic attack was injected into normal individuals. Polyarthritis developed in the recipient. When the blood of this recipient was then reinjected into another group of normal individuals, a milder attack of polyarthritis resulted.

QUESTION: It is definite from your figures, that rheumatic fever is transmitted with a certain gene. Can we say that in certain families a gene is labeled as rheumatic and that this will manifest itself in the progeny of this family as rheumatic fever?

DR. WILSON: Yes. That is the assumption for recessive inheritance. However, the disease is not transmitted, but only the susceptibility to the disease.

QUESTION: Have we a right to say that once a rheumatic child is discovered in a family, and the parents are known as being normal, that both of these parents must be genetic carriers?

DR. WILSON: Yes. That is the assumption on the postulate of recessive inheritance.

QUESTION: Have any studies been made to show why rheumatic fever has a low incidence among higher income groups? Is there any genetic explanation for the geographical distribution of rheumatic fever?

DR. WILSON: These questions cannot be answered at the present time, since we do not have factual data on either group. The second question could easily be answered by taking such groups of genetically susceptible individuals and transporting them to other climates to determine whether predictions would be realized. Genetic factors would not be expected to vary in different geographical areas, although the frequency of cases may.

QUESTION: Do you assume that if both parents have rheumatic disease, that all their progeny will have rheumatic disease?

DR. WILSON: One hundred per cent will be genetically susceptible to this disease and it is probably that one hundred per cent will develop it, at least in New York City.

QUESTION: Do you believe, on the basis of your observation that when susceptible children are transported to a subtropical climate before rheumatic disease has manifested itself, that the incidence will be the same?

DR. WILSON: I do not know. That remains

to be shown. Several such studies have been initiated.

QUESTION: How does this theory of genetics as related to rheumatic disease take into consideration the varied distribution of this disease in various locations in the same city?

DR. WILSON: I do not believe that we have any careful studies to support the premise of significant difference in distribution in various localities.

QUESTION: I believe that in a study in New Haven it was shown that rheumatic disease is distributed along the river front. How do you explain that?

DR. WILSON: River fronts usually have congested populations and many hospitals and clinics, which may well account for increased frequency of rheumatic fever in such areas.

QUESTION: In a previous session, we were shown that a milk-borne epidemic of hemolytic streptococcal upper respiratory infections was followed by an epidemic of rheumatic fever. How does this fit in with the genetic explanation?

DR. WILSON: We have to assume that in this group there were a great number of susceptibles. There are many epidemics of scarlet fever recorded without epidemics of rheumatic fever following them.

QUESTION: How can we explain a seasonable variation in the incidence of rheumatic disease on the basis of your concept?

DR. WILSON: As a matter of fact, a close analysis over many years shows that the incidence of rheumatic recurrences did not vary much from season to season. It is true that in the summer months in the City of New York the incidence of rheumatic disease is low. On the other hand, exceptions to the rule occur. This summer, for instance, we had a high incidence. It is a common experience that recurrences are apt to follow exposure and chilling. You take, for example, a child from one of the inactive buildings here and let him go out into the rain and get his stockings wet, and he may get a recurrence.

QUESTION: Can we say that that same child would develop a recurrence without wet stockings?

DR. WILSON: He might.

QUESTION: If in your opinion the hemolytic streptococcus does not play an important role in the cause of rheumatic fever, then chemotherapy would not prevent rheumatic recurrences. May I ask you to give an explanation of the following occurrence?

The child came to the clinic several years with a history of chorea of five years' duration. Between the fifth and the sixth year, the child received chemotherapy in prophylactic doses. This year is the only year in which the child did not have any chorea.

DR. WILSON: You cannot be certain that chemotherapy prevented a recurrent attack of chorea. Chorea is a self-limited disease with varying years of freedom from recurrence.

QUESTION: Would you repeat the therapy?

DR. WILSON: No. I would not have given it in the first place. I know that we like to do something for the patient but we must keep our two feet on the ground. Until it can be definitely shown that prophylactic chemotherapy prevents recurrence, it is inadvisable to use a drug which may in some instances have deleterious effects. Some years back, the panacea for rheumatic recurrences was tonsillectomy. Every child had its tonsils removed. We soon found out that it made no difference in the incidence of rheumatic recurrences. This form of therapy was therefore given up.

QUESTION: Would you say that infection may be an added factor in the causation of rheumatic disease?

DR. WILSON: I do not know. But it is certainly worth while to find out. We now have a tool which we can use. The risk of rheu-

matic recurrences is worked out carefully and if we apply this risk to the study of the incidence of rheumatic recurrences in patients receiving prophylactic chemotherapy, we might get the evidence.

QUESTION: Rheumatic disease is, in the eyes of the clinicians, an illness which behaves very much like an infection. The clinician further feels that we now have certain drugs which are universal bacterial killers, such as penicillin, and sulfonamide derivatives. Would you not be willing to use these universal killers on the basis that the disease behaves clinically like an infection?

DR. WILSON: If the proper study were made, it would serve to end the doubts in this direction but no such study has yet been done to meet requirements for adequate statistical analysis. I believe that if these drugs are to be used, they are to be used experimentally only. In addition, we must be certain that these drugs do not have harmful effects. Certainly, as far as sulfon therapy is concerned, enough harmful effects have been observed to cause the army and navy to discontinue its routine prophylactic use.

It is admitted that the literature gives the general practitioner a feeling of confidence in the use of sulfon therapy as a prophylaxis of rheumatic disease. None of the evidence I have shown before indicates that sulfon prophylaxis prevented rheumatic recurrences. Until we know more about the factor or group of factors which may be responsible for the manifestations of rheumatic disease in a susceptible individual, we should limit prophylactic chemotherapy to experimental studies.

QUESTION: How do you explain racial difference in the incidence of rheumatic disease? We were told, for instance, that rheumatic disease is less common among the colored people?

DR. WILSON: I do not believe that adequate studies have been published to support this observation. It depends on where you are studying these cases. In the hospitals where one racial or religious group predominates, there will be a greater incidence of rheumatic disease within that group.

QUESTION: Do you advise a positive by positive mating not to have children?

DR. WILSON: I would explain the risk to them carefully, and leave it to their judgment.

QUESTION: How about a positive by negative mating?

DR. WILSON: I would tell them that if the negative side of the family is free from rheumatic fever, the chances are small that any of the offspring will have rheumatic disease. If, however, close relatives of the negative parent are rheumatic, I once again would explain the risk and leave it to their judgment.

QUESTION: It has been said that children grow up to become rheumatic. In other words, rheumatic disease manifests itself not at birth, but several years after birth. Is there any explanation why in the same family, under the same environment, with the same rheumatic background, one child should manifest the disease at six years of age and the other one at thirteen years of age?

DR. WILSON: We cannot predict as yet which child in the family is going to get rheumatic disease and at what age, but we can definitely state what proportion in a given family with a given background, will develop rheumatic disease at each specific age. For the present, we have no explanation for the age incidence.

Combined Staff Clinics

Lymphomas

THESE are stenotyped reports of combined staff clinics of the College of Physicians and Surgeons, Columbia University. The clinics, designed to integrate basic mechanisms of disease with problems of diagnosis and treatment, are conducted under the auspices of the Department of Medicine. The reports are edited by Dr. Frederick K. Heath.

DR. JOSEPH C. TURNER: The central part of this morning's discussion on the lymphomas and recent advances in their therapy will be undertaken by representatives of the Department of Radiology and the Department of Pharmacology. But meanwhile, before introducing these investigators, I should like to recount briefly certain studies that have occupied workers interested in the field for some years, and to take note of what progress has been made.

The lymphomas, you will recall, constitute a group of tumors marked by prominent involvement of the spleen and lymph nodes. The term itself is merely a convenient one for the use of both clinician and pathologist, and should carry no connotation regarding the fundamental relations of these diseases to one another.

We include among the lymphomas the leukemias, Hodgkin's disease, the apparently true tumors of the lymph nodes—lymphosarcoma and reticulum-cell sarcoma—as well as the somewhat unusual, so-called giant follicular type, which runs a somewhat more benign course than the others, but in time, like them, becomes what is evidently malignant disease.

From the time that Sternberg first described accurately the characteristic pathology of Hodgkin's disease there have been persistent and patient efforts to remove this disorder from the neoplasms and to identify it as an infectious disease. There have been many reasons for this, notable among them the histological picture, for, in contrast to the general run of tumors, Hodgkin's disease is marked not by the proliferation of a single type of cell but rather by the appearance of a pleomorphic growth. Included in the lesions are reticulum cells, as well as eosinophils and giant cells and there is usually considerable proliferation of fibrous tissue, too. Thus the histological features suggest some sort of infectious granuloma.

At first the belief was widely held that this might represent an unusual form of tuberculosis, because tuberculosis is found with unusual frequency in Hodgkin's disease. Some autopsy figures give an incidence of active tuberculosis as high as 20 per cent in classical Hodgkin's disease. This is perhaps twice what might be expected. But it appears that the tubercle bacillus is probably a secondary invader, assuming a heightened degree of virulence presumable because of some alteration in the resistancy of the host occurring in consequence of Hodgkin's disease. Numerous attempts have been made to recover tubercle bacilli from the lymph nodes and spleen of Hodgkin's disease but not more, I think, than perhaps 10 per cent of the cases show acid-fast bacilli either on section or by guinea pig inoculation.

From 1910 to 1920, there were reports of the cultivation of bacteria of various types, especially diphtheroids, from Hodgkin's disease. More recently, the claim has been made that Brucella organisms may be recovered with great regularity. It seems

unlikely that any of these infectious agents has etiological significance.

Between 1920 and 1930, an extensive program of bacteriological investigation was undertaken in England under the aegis of Dr. Mervyn Gordon and the findings were later published as "The Rose Research in Lymphadenoma." Gordon and his colleagues undertook to repeat all the bacteriological investigations of the past, looking for tubercle bacilli, spirochetes, fungi, etc. Their most notable contribution was the discovery that the lymph nodes in Hodgkin's disease contain a factor which is capable of producing encephalitis in rabbits. It now appeared for the first time that this disease might be transmitted to an experimental animal and could be caused by a virus. Unfortunately, however, further study of this agent of Gordon showed that it was not truly infectious since it could not be transmitted from one animal to another in series. Moreover, it was finally shown that the agent was not specific for Hodgkin's disease but was associated rather with the eosinophil, whether found in the lesions of Hodgkin's disease or in normal tissue. So that, although the past forty years and more have seen a number of attempts to identify Hodgkin's disease as a bacterial or a viral infection, none has succeeded and the question remains open.

The paramount difficulty, of course, has been the failure to reproduce the disease in an experimental animal. This consideration leads us to direct our attention briefly to what may fairly be said to be the most notable recent advance for the study of lymphoma, namely, the development of strains of animals with spontaneous leukemia.

The earliest systematic work on the subject was done by Ellermann about 1908, when he showed that the erythroleukosis of fowls could be transmitted from one animal to another, and furthermore, that he could recover a filtrable agent which was capable of reproducing the disease. Since then this is recognized as a disease due to a filtrable virus and, as you know, there are several other bird tumors that represent viral infections.

In 1929, Richter and McDowell, by inbreeding, produced a leukemic strain of mice. There was now available in the field of mammalian lymphoma an experimental animal which could be subjected to controlled investigations. Since that time there has been a great deal of work done with mouse leukemia and there are now a number of strains which develop spontaneous lymphatic leukemia or lymphosarcoma, as well as myeloid types of leukemia. The animal disease resembles very closely the human one. Smears of bone marrow and peripheral blood, as well as sections of the various affected organs, look so much like the counterparts in the human that they are virtually indistinguishable.

Opportunity has been taken in the past decade to investigate the influences of genetic pattern, of mother's milk and of numerous other factors on the development of animal leukemia. It has been possible also to make certain biochemical observations, including the comparative metabolic behavior of tumor cells, the effects of hormones and therapeutic agents on the tumor, and so on. These advances emphasize once again the importance of the experimental animal in the special problem of cancer.

STUDENT: Have any biological tests for lymphoma been developed that can be applied clinically?

DR. TURNER: Unfortunately, no. The diagnosis still rests on biopsy of affected tissue, usually superficial lymph nodes. In this respect, the most important rule to be observed is: in any non-leukemic case of lymphoma, biopsy must be done before therapy is undertaken. Mistreatment of tuberculosis and other infectious granulomas,

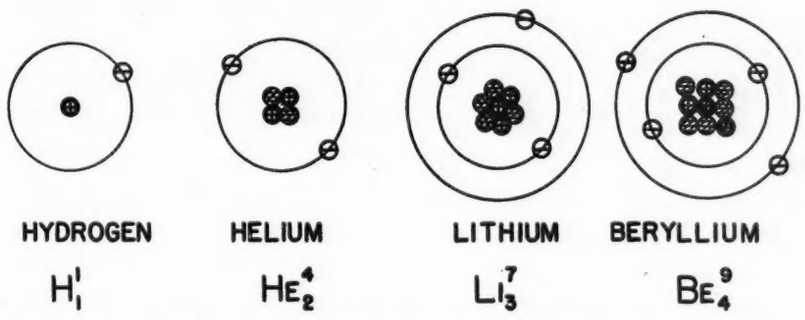


FIG. 1. Showing the "solar system" concept of the atom. Note the orbital electrons and the nucleus made up of protons (dark) and neutrons (light).

which may be indistinguishable from lymphoma clinically, is otherwise inevitable. Exceptions to this rule should be made only when biopsy is impracticable, as, for example, with a mediastinal lymphoma in a patient presenting no palpable superficial nodes in the neck or elsewhere.

We are fortunate this morning in having Dr. Quimby of the Department of Radiology to tell us something about radioactive isotopes, which have been introduced within the past ten years for the treatment of malignant disease. Perhaps their most notable success has come in the field of lymphoma.

DR. EDITH QUIMBY: Radioactive isotopes should not be considered by themselves in discussing the treatment of any disease but rather they should be regarded simply as one source of radiation; therefore, the possibilities of the whole field of radiation therapy should be considered in any such discussion. What are the radiations which are available and usable? Where are they obtained and how are they controlled? How do they produce the effects which are observed?

In the treatment of the lymphomas the two types of radiation which are used are *x*-rays and the radiation from artificially radioactive substances. As to *x*-rays, your reaction to them is probably that they come out of *x*-ray tubes and are under the control of the radiologist; he knows how they should be used and you will consult with him—and that is probably all right. As for the artificially radioactive substances, in a few years you may be just as blasé. You may believe that they come in a bottle with the specified dosage and you give them; but that, at the present time, is not the case. Because these are such new substances and because there are very few specialists in their use, it seems profitable to discuss their origin and nature briefly.

In order to do that, one must begin with atoms (since these are the elementary particles of matter from which the radiations come), and with the present day "solar system" concept of the structure of the atom. This concept of atoms (Fig. 1) supposes that each consists of a positively charged nucleus, which carries essentially all the weight of the atom, and a system of orbital electrons, each electron having a negative charge, with just enough electrons to balance the positive charge of the nucleus. This discussion is concerned only with nuclei.

Artificially radioactive substances are substances whose nuclei are in an unstable condition. Nuclei of atoms are built up of two kinds of particles, each having about the same mass: a particle which has no charge and which is called a *neutron*, and a particle

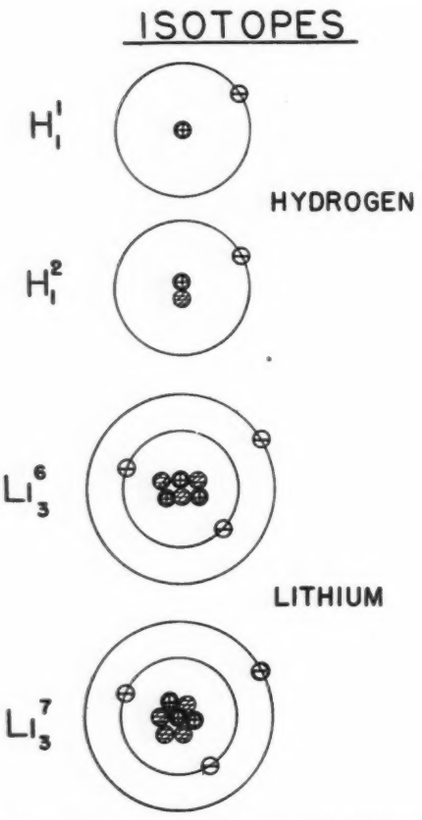


FIG. 2. Isotopes of hydrogen and lithium containing an extra neutron in each nucleus.

which has a unit positive charge and is called a *proton*.

In proceeding from element to element in the periodic system, the number of protons is increased by one. Hydrogen, the lightest element, has one proton in its nucleus, helium has two, lithium three, beryllium four, and so on. The number of protons in the nucleus is the *atomic number* of the substance; it is that which determines its chemical nature. For every number from 1 to 94 there is an element, and that element is defined by its atomic number.

The number of neutrons in the nucleus varies. It may be the same as the number of protons or greater to almost twice as many. The sum of neutron and proton numbers is the *atomic weight*. The symbols for the elements now are written not only with the customary chemical abbreviation but with a subscript which gives atomic number and a superscript which indicates atomic weight, thus defining the element exactly.

As I said, the atomic number is fixed for each element but the atomic weight may be variable. Some elements have atoms of only one atomic weight and others have as many as seven or eight or even more different atomic weights for one atomic number. This is due to varying numbers of neutrons with the same number of protons.

Thus (Fig. 2) there are the two types of hydrogen: ordinary hydrogen, the nucleus of which is just a proton, and heavy hydrogen with a proton and a neutron. Atoms having the same atomic number and different atomic weights are called *isotopes*.

The lighter atoms have simple nuclei. In ascending the atomic scale, nuclei contain more neutrons and more protons and become more and more complicated. They are not rigid assemblages of particles. There are within the nuclei forces and activities going on which render these complicated nuclei unstable. Such nuclear instability means that at some time a nucleus will no longer be able to maintain itself the way it was but will react by ejecting a small part of itself. This phenomenon we call *radioactivity*. All elements with atomic numbers above 83 are naturally radioactive, that is, at some time during their existence such radioactive atoms become unstable, expel a small portion of themselves, (which may be, in the case of the naturally radioactive substances, a group of two neutrons and two protons) and settle down as atoms of different elements.

Artificial radioactivity is the making of atoms which are normally stable, unstable in the same way as naturally radioactive atoms exist in the state of nature. This can be accomplished by shooting into such a stable nucleus an extra particle, thereby putting it in a strained state. That extra

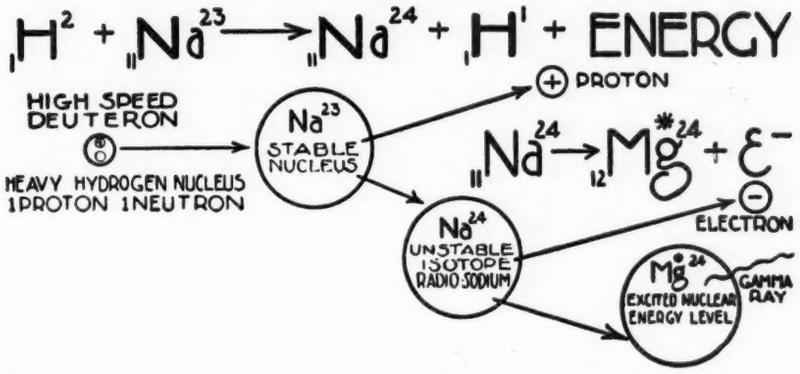


FIG. 3. Indicating the production of artificial radioactive sodium by bombarding normal sodium with the nuclei of heavy hydrogen.

particle may be a neutron or a proton, a deuteron (the nucleus of heavy hydrogen), an alpha particle from naturally radioactive material or possibly some other small agglomeration of nuclear particles. What happens then is that the new nucleus, the one with the extra material in it, is unstable and disintegrates in the same manner as naturally radioactive substances do.

One characteristic which it is important to remember about radioactive substances is that, although the disintegration of any particular atom is a completely haphazard affair, statistically, for any particular radioactive substance, half of all the atoms present will have disintegrated during a certain period; during a subsequent equal period half of what is left will disintegrate, and in another period half of that, ad infinitum. Therefore, by means of a simple mathematical expression, one can always calculate how much material should be left if it is known what was started with, or one can always calculate what should have been present in the beginning if it is known what is present at any particular time.* These half-lives, as they are called, vary for different substances over an enormous range, from fractions of a second to millions of years. For the artificially radioactive substances which have become useful in experimental or therapeutic work, the half-lives are of the order of days or weeks, or in a few cases, of a few months or years.

Figure 3 represents a typical example of the sort of thing that happens. This is the manufacture and disintegration of radioactive sodium. Ordinary stable sodium is bombarded in a cyclotron with the nuclei of heavy hydrogen, deuterons. The deuteron goes into the sodium nucleus, apparently breaks apart and the proton comes out. The neutron stays in, therefore making an unstable sodium having an atomic weight of 24, one more than normal sodium. This cannot exist the way it is, so one of the neutrons inside breaks down into a protron and an electron. The electron is expelled. That means that the substance now has 24 positive charges instead of 23. It is not sodium any more but is magnesium. In settling down to its normal state, the magnesium expels a gamma ray. Thus radioactive sodium gives out a beta ray (an electron), and a gamma ray in the course of its disintegration. So for the purposes of therapy there are available in addition to the *x*-rays, the beta and gamma radiations from certain radioactive substances.

How can these substances produce reactions in the living tissues? What is the phe-

* The equation is: $Q = Q_0 e^{-kt}$ where
Q is the amount at future time t days hence
Q_0 is the amount of substance at present time
e is the base of natural logarithms
k is the decay constant per day and may be obtained from the relation $50 = 100\ e^{-(k \times \text{half period})}$

nomenon by which energy is transferred from the radiation to the matter? The only way that anything can be done, as far as is known, in this universe is by the use of energy or the transfer of energy from the doer to the recipient. The way energy is transferred from radiation to matter is by a process called *ionization*. This is not the sort of ionization which occurs in chemistry—the breaking apart of molecules into positive and negative ions. This is the removal of an electron from the atom, leaving the atom in a charged state. If an electron is taken out of an atom there is left an atom with too few electrons to balance the nucleus. That is a positive ion. The electron itself or anything to which it may attach itself is the negative ion. The state of ionization lasts very briefly, but during that state abnormal or unusual atomic groupings may occur. For instance, if the atom were a part of a molecule and the electron pulled out was one of the binding electrons, the whole molecule might well break down. It is during the process of ionization that the changes come about which set in motion the ultimate biological change. From there on very little is known about what happens. That is one of the fields in radiobiology which must be investigated a great deal more.

The sort of track which the ionizing particle makes depends largely on its speed and energy. There may be a lot of ions crowded close together in a short path, or they may be spread out along a longer track, depending upon the speed of the particle, the energy of the *x*-ray or the speed of the beta ray which does the ionizing. If there is intense ionization, crowded together in one or two cells, a different result may be expected from that where the same number of ions that spread out over a great many cells. What steps occur after ionization to bring about biological changes is not known.

What are the final results in the cell? They are things which can actually be observed: increased acidity of protoplasm, increased permeability of membranes, clumping of nuclei, breaking of chromosomes, halting of cell division, and finally, entire stoppage of all cell activity.

A little radiation may not produce any visible effect or it may produce some effect in some cells. A little more produces more effect, just like any other kind of drug, until finally an irreversible change results. If all living cells responded in the same way to radiation, it might be impossible to bring about any action upon specific cells. However, even in cells that are all alike some respond more readily than others. In cells that are different there are groups of cells which are much more radiosensitive than others. Fortunately, the lymphoid cells are the most radiosensitive of all. Following them are the epithelial and endothelial cells, and then connective tissue, bone, fat and nerve, in that order. Therefore, if radiation is poured into a mixed kind of tissue, a greater reaction may be expected in lymphoid tissue than in any other, and that of course is desirable in the treatment of disease of lymphoid cells.

In the diseases with which we are concerned, one may want to treat just a local growth, either lymph nodes, enlarged spleen, or something of that sort, or one may want to give a generalized irradiation. When the treatment is to be by means of *x*-rays, the treatment is adapted to each patient. It may be administered over the diseased areas, over blood-forming areas, or over the whole body, depending upon the radiologist's idea in the beginning and the patient's response as therapy goes on. If a great deal of the body is involved, not nearly as much radiation to any particular region can be tolerated. In this connection, the measurement of *x*-rays becomes important.

The unit of *x*-ray dosage is the *roentgen* and it is a queer kind of a unit. It is in essence the amount of radiation which produces a

certain number of ions in every gram of tissue on which this beam of radiation falls. A skin dose of 100 roentgens means that every cu. cm. of skin, and immediately underlying tissue, will be ionized to that extent; the tissues lying below the skin will be less ionized because some radiation will be absorbed. If a very small field in the skin is irradiated, then not very much of the body will be absorbing energy; the effect on the system as a whole will not be much. But if there is a big field on the skin, although the dose is still 100 r, the amount of absorption by the patient is very much more. Therefore, the size of the irradiation field is very important in considering dosage in *x*-ray therapy. If just a small field is used, in the course of a month a dose of several thousand roentgens may be given to that skin. Over a large field only a few hundred may be given and over the whole body less than one hundred can be used. That must be borne in mind in planning treatment. The sort of treatment given by the artificially radioactive substances is different. That is a whole body irradiation.

We cannot go into the matter of dosage calculations with these isotopes except to say that there is a formula by means of which these doses can be related to *x*-ray doses. The formula is very simple:

$$\text{e. r.} = 0.088\ VT$$

It simply says that when one microcurie of radioactive isotope is deposited in 1 Gm. of tissue and remains there until its total disintegration, the "effective roentgens" or "equivalent roentgens" are equal to 0.088 times its half life in days times the energy of the beta particle in electron kilovolts. It is obvious that the longer the life for a given amount of material the greater will be the dose. Figuring that out for phosphorus and sodium, it will be seen that per millicurie of sodium the "e. r." will be much less than for phosphorus, since the life time is so different. The beta ray energies are about the same. Sodium also emits gamma rays and this must be taken into account when thinking about dosage. Thus 25 millicuries of sodium produce about the same effect as 2.5 millicuries of phosphorus. It is evident that dosage cannot be calculated in millicuries without knowing about the life time and the radiations of the substance used.

Dr. Henry Aranow, Jr.: It might be interesting if we had an explanation of what the millicurie is.

Dr. Quimby: The millicurie is that amount of any radioactive material such that 3.7×10^7 atoms disintegrate per second. A microcurie is $\frac{1}{1000}$ of that. A curie is 1000 times that. Initially the curie was the amount of radon which was produced by a gram of radium when the two were in equilibrium, and the unit has been taken over now to all artificially radioactive as well as naturally radioactive substances. It therefore is no measure of the total weight of material administered. If you have a curie (or millicurie) of a very short-lived material, you will get 3.7×10^{10} (or 3.7×10^7) disintegrations at that instant, but the decrease in activity will be very rapid; while if you have a long-lived material you get the same activity at the instant and the decrease will be very slow. The result of that, as I said, is you can give a considerable number of millicuries of a very short-lived material but you have to be careful about giving very many millicuries of an element which is going to stay in the system for a long time.

At the University of California, radioactive phosphorus was one of the first substances made in any great quantity and therefore the substance investigated most assiduously. Among the studies made was the manner in which it was distributed through the living organism. It was found that radioactive phosphorus was deposited to a slightly greater extent in bone and bone

marrow, spleen and liver than it was in other tissues, not a great differential but one that could be noted.

That made Dr. Lawrence and his associates think that possibly by giving a dose of radioactive phosphorus which could be tolerated by the whole body, a differential radiation could be given to leukemic or lymphomatous cells. They started very cautiously with some leukemic patients and were very pleased with the results. The white counts went down, the platelet counts went up. Sometimes the spleen decreased in size. Symptomatically, the patients were much better. So they started to treat a series with planned therapy. Since then a number of other institutions have done the same sort of thing. The idea is this: The patient is given a dose of a phosphate in which a certain amount of the phosphorus is radioactive. This phosphorus then distributes throughout the body just as all phosphorus does. That means that radioactive atoms are incorporated in cells all through the body, just like normal phosphorus. They are normal phosphorus for all purposes as long as they stay phosphorus but every one of these atoms explodes at some time. In exploding, it shoots out beta particles which go through the cell in which it is deposited, and two or three adjoining cells. That irradiation goes on gradually, decreasing as the phosphorus is used up.

One millicurie of phosphorus distributed throughout the body means that 37 million phosphorus atoms are exploding every second. That sounds like a very big number until it is realized that 1 Gm. of tissue contains approximately one hundred billion billion atoms. Then 37 million does not seem so many. Of course explosions at that rate do not keep up. They get fewer as the phosphorus is used up at a rate of about 5 per cent a day, half in two weeks, half of what is left in two weeks, and so on. The patient is subjected to a gradually decreasing universal bath of radiation.

In the spring of 1946 the group at St. Louis, Reinhart, Moore, Birnbaum and Moore, made a complete review of everything they could find in the literature on the use of radioactive phosphorus in this diseases and published it in the February, 1946 issue of the Journal of Laboratory and Clinical Medicine. Anyone who is interested in this subject should look over this report. It is very complete.

I will simply read you their conclusions. In myelogenous leukemia, there were 107 cases in the literature plus thirty-nine of their own. In the chronic form, all symptoms were relieved in 50 per cent; most of the symptoms in 85 per cent. Splenomegaly disappeared or was reduced in 64 per cent. They conclude that no cures and not even much relief was produced in acute and subacute cases. In chronic cases they made a comparison between the results of radioactive phosphorus, *x*-rays and Fowler's solution, and decided that the remissions produced were about the same. However, they say that therapy with radioactive phosphorus is pleasanter for the patient and there are no undesirable side effects—nothing like radiation sickness or toxic reactions which sometimes are produced by Fowler's solution. Therefore, only from that point of view do they consider it preferable.

In lymphatic leukemia there were 120 cases in the literature plus forty-five of their own. Of these eighty-four were chronic and seventy-one acute. They conclude that symptomatic relief in the chronic form is about the same as for the myelogenous type and that there is no help in acute lymphatic leukemia. They had no remission in their own group longer than one year; that is, after a year they had to give more treatment. The results are about the same as with *x*-rays. The treatment with radioactive

phosphorus is sometimes useful after *x*-rays have failed.

They report scattered results for other lymphomas. There are some specially good results in lymphosarcoma and particularly in reticulum-cell sarcoma. In Hodgkin's disease they report no improvement over *x*-rays. The series is very small and hard to evaluate.

Some time ago we were using radioactive sodium for some other purposes and the question arose: Could we not treat leukemia with that? Radioactive sodium is distributed uniformly through the extra-cellular body fluids. Therefore, a person treated with radioactive sodium would have a more homogeneous type of irradiation than with phosphorus. That is not any particular advantage. However, the life time of radioactive sodium is fifteen hours instead of fourteen days. That means that treatment could be better controlled because reactions could be observed more rapidly and doses followed more closely. A few patients have been treated and Dr. Evans will tell you the results. The series is not as large as the series for phosphorus. We did not expect any better results than with phosphorus but felt they should be as good.

There is just one other point which I should like to mention and that is the fact that these radiations are no respecters of persons. They would just as soon damage you as your patient if you give them a chance. Radiologists have had a horrible example for many years of what happened to the early radiologists who were not properly protected, and they are not going to get into trouble with these elements if they can help it. But there are a lot of people now coming into the field of radiation research and nuclear research who do not have that horrible example in the background. These little glasses of solution look very harmless. It is hard to believe if we pick up a jar of such material and carry it around for a few minutes we may in the course of a couple of months have some very sore fingers. With beta ray products such as phosphorus, protection is relatively easy. An amount of lead or lead equivalent equal to about a millimeter will stop most radiations. But with gamma ray products the story is different. The gamma rays are extremely penetrating, and if any quantity is to be handled, before starting any routine, the only recommendation which I can give at the present time is to get in touch with the radiation physicist who can advise on necessary precautions. We certainly do not want any such tragedies forty years from now, as there were among people who forty years ago handled radium injudiciously.

DR. TURNER: I think that before asking for questions on the subject outlined by Dr. Quimby we might hear from Dr. Evans, who has had an intimate experience in the past few years with the treatment of patients with these agents.

DR. TITUS C. EVANS: Dr. Quimby and I have been working with Dr. Lenz on a study of the effects of radioactive sodium on patients with chronic leukemia. The patient drinks the radioactive sodium combined as sodium chloride in a very dilute solution which is practically tasteless. Within a short time the sodium is distributed throughout the blood and extra-cellular fluids. Organs containing much blood or other fluid containing sodium may receive slightly more radiation than the body generally but the general distribution of the sodium produces whole body radiation. Success, therefore, depends primarily upon the malignant cells being more radio-sensitive than normal cells. Since the half-life of radioactive sodium is about fifteen hours, a single dose will irradiate a patient with decreasing intensity over a period of two to three days. Loss of effective radiation through elimination is negligible. The radioactive sodium is measured in millicuries (mc) and the

amount administered each time depends upon body weight and condition of the patient.

We have treated two chronic lymphogenous leukemia cases which have been well controlled for approximately a year. The two cases were similar so I will discuss only one. The white blood count before treatment was approximately 70,000 and a small dose (10 mc) brought the count down to about 35,000 within a few days. After a week the count increased again, but another treatment brought the white blood count down to within normal limits. We have been able to keep the count below 20,000 for well over a year by a treatment every two or three months. Each treatment has been followed by improvement in the differential and an increase in the number of platelets.

The next patient to be discussed has chronic myelogenous leukemia. Before treatment the white blood cells were about 275,000. The spleen and liver were enlarged. Doses were increased from 15 mc to 35 mc before obtaining a drop in the count. The count is now within the normal range and the spleen and liver are no longer palpable. We have not observed any harmful effects of the radiation and these patients have suffered no discomfort.

One case of myelogenous leukemia has been more difficult to control. Dr Lenz had been able to control the disease for about a year with fairly heavy doses of *x*-rays. *X*-radiation in amounts necessary to be effective caused the patient considerable discomfort. We were able to bring the count down time after time with radioactive sodium without producing any ill effects. Regressions were only temporary and the spleen was gradually increasing in size, so that much heavier treatment was necessary to reduce the white blood count and the spleen size to normal. This amount of radiation did cause the patient to feel uncomfortable for a while. The patient says that the reaction was not as great as he had experienced with even moderate amounts of *x*-radiation. This is a rather stubborn case and I do not know how long it will be possible to continue treatment.

I will mention another case of myelogenous leukemia in which the results of treatment have not been too encouraging. This was a little girl of six in poor physical condition, and it was inadvisable to use heavy doses because of her anemia and low platelet count. It was possible to keep her fairly comfortable for about a year and also to obtain some improvement in the blood picture. The spleen, however, continued to enlarge, and she continued to grow weaker. At autopsy the spleen was found to be packed with malignant cells.

We have attempted therapy, with discouraging results, in terminal Hodgkin's disease, terminal lymphosarcoma, terminal lymphogenous leukemia and acute lymphogenous leukemia in children. In these cases some relief of pain and some improvement in blood picture was obtained but the progress of the disease was not affected.

One case of polycythemia vera has been studied. The patient was treated at weekly intervals for almost two months and has not been treated since that time (now over a year). Within three months after beginning treatment, the red blood cells had dropped from 8,000,000 to less than 5,000,000 and the hemoglobin had been reduced from 25 Gm. to less than 14 Gm. The blood picture has remained essentially normal up to the present time.

Use of radioactive sodium offers some advantages over *x*-ray treatment and possibly over radioactive phosphorus therapy. The use of radioactive sodium in therapy is limited, because of its short half-life, to institutions near the source of supply. Also, care must be exercised to avoid overexposure of the patient and the attending personnel.

DR. TURNER: We might pause here for a moment to ask if there are any questions about what Dr. Quimby and Dr. Evans said. I should like to ask one question and that is to what extent can a biochemical account be given of the effect of *x*-rays?

DR. QUIMBY: Perhaps some of the biochemists can answer that better than I can.

DR. TURNER: There has been increasing interest in the effect, for example, of chemotherapeutic agents on substrate competitors, enzyme inhibitors, and so forth and so on. Is there any information of that kind available with respect to the influence of *x*-rays?

DR. DEWITT STETTEN, JR.: Dr. Quimby, I do not have information on this point.

DR. EVANS: There have been attempts to pin down the fundamental effects of *x*-rays to enzymes breaking down large protein molecules to small ones, producing toxic substances, etc. Apparently such changes take place but to a limited extent, and so many reactions occur at the same time that it is difficult to narrow the effect to any one thing. Enzymes have been thought to be sensitive to radiation, as they are under certain conditions, but in the cell it is a different problem. It is difficult to make a chemical study of this kind on a biological basis, although we can try it.

A DOCTOR: Is there any evidence that these radioactive elements can be so chemically combined as to increase the differential of absorption?

DR. QUIMBY: That is one of the things on which a great deal of work is being done. At the present time there has not been much success but many people are trying to find some means of making substances localize in particular types of tissue and in particular types of organs. If that is ever done, of course, there will be the possibility of local radiation from these substances. At the present the only example is iodine, which will localize in the functioning thyroid gland. If we can make other substances do the same sort of thing for other regions, it will be fine.

DR. TURNER: We will go on to the subject of the chemotherapy of lymphomas. As you all know, Fowler's solution is one of the oldest chemotherapeutic agents which has stood the test of time. It has been employed in the treatment of leukemia and other types of lymphoma for about eighty years with such success that at times its usefulness approximates that of *x*-ray. It has been largely given up, however, because of untoward side reactions such as nausea, vomiting and diarrhea. One is not able to predict as satisfactorily that a therapeutic response will be obtained, whereas with *x*-ray therapy we are pretty familiar with what may be expected.

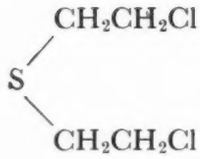
More recently, substances such as benzol and urethane have also been employed in the treatment of leukemias. Among the most interesting compounds which have been developed, largely as the result of work done during the war, are the nitrogen mustards, which have been used for perhaps three or four years.

Dr. Gilman of the Department of Pharmacology has been good enough to come up and tell us about some of the work on the nitrogen mustards.

DR. ALFRED GILMAN: I regret that the discussion on the nitrogen mustards was introduced as relating to the chemotherapy of lymphoma. Rather I think the contribution that these compounds have made to date is to point to fundamental mechanisms involving the cell nucleus, which are shared by *x*-ray, radioactive isotopes and the nitrogen mustards; the last mentioned, as far as we know, possess no type of radioactivity.

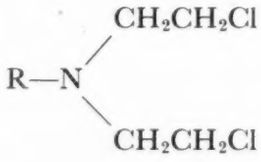
I would like to trace the story of the nitrogen mustards back to World War I, when sulfur mustard was so widely employed as a war gas.

Sulfur mustard has the following structure:

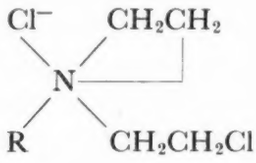


Sulfur mustard reacts with water eventually to replace the chlorine with hydroxyl, with the release of HCl. During World War I it was presumed that the effective action of mustard gas was due to the release of small amounts of hydrochloric acid within the cell. In retrospect it is difficult to imagine how such a theory could have been entertained, because the small amount of hydrochloric acid released would most certainly be readily neutralized and buffered by the cell. A few of the more astute observers noted that following extensive mustard lesions of the lungs, leukopenia often developed and was a grave prognostic sign. However, this observation was largely ignored, and between wars practically no studies were conducted on the effects of mustard on hemopoietic tissue.

With the advent of World War II, a new series of compounds was introduced, the nitrogen mustards, which differed only in that nitrogen replaced sulfur. Inasmuch as nitrogen is trivalent, there is room for one substituent group in the molecule. Following is the basic structure of the series of nitrogen mustards in which R represents the substituent group:



There was a background for appreciation of the chemical behavior of the beta-chloroethylamines because these compounds had been extensively studied. For example, it was known that in aqueous solution the beta-chloroethylamines undergo intramolecular cyclization as a result of a ring clouser between the beta carbon and the nitrogen to form a quaternary ammonium base:



Whereas the original beta-chloroethyl group is very unreactive, the ethyleneimine group which is formed is one of the most reactive of organic structures. For example, it will react with a variety of biologically important chemical groupings such as amino, carboxyl, sulfhydryl, sulfide, phenolic, imidazole, organic phosphate, etc. In fact, any attempt to define the basic mechanism of action of the nitrogen mustards on the basis of chemical affinities is thwarted by the wealth of possible explanations.

The chemical reactivity of the ethyleneimine ring with biological compounds is of particular interest for the reason that the biological effects of the nitrogen mustards parallel and are almost identical in every respect with the biological effects of radiant energy. To emphasize this point, let us review briefly the effects of the nitrogen mustards on unicellular organisms and on the mammal.

Following the intravenous administration of nitrogen mustards in mammals, the first effects are noted in lymphoid tissue. In experimental animals one observes almost complete dissolution of lymphoid tissue within twenty-four hours. This is reflected in the peripheral blood by a severe lymphopenia. Similarly in human subjects following the injection of a few doses of nitrogen mustard, one may observe an absolute lymphopenia in the peripheral blood. There is also an effect on granulocytes, the degree of which is related to dosage. With a few doses of 0.1 mg. per kg. daily, one observes a moderate granulocytopenia. Increasing the number of doses results in a

more severe granulocytopenia. With ten daily doses the bone marrow is depressed to the extent that only occasional granulocytes are observed in the peripheral blood. Likewise, platelet formation is depressed. Again, the degree of thrombocytopenia is related to dosage. If one further increases the dose of nitrogen mustards in experimental animals, the effects now extend to the epithelial tissue of the gastrointestinal tract. Degenerative changes occur which eventually lead to a severe hemorrhagic enteritis. With still higher doses, there is evidence that every cell in the body is being affected. Animals run a progressive downhill course. Disturbances in water and electrolyte metabolism are outstanding. Severe diarrhea and vomiting contribute to anhydremia and circulatory collapse. In addition, the permeability of the cell membrane appears to be affected in that potassium is lost from and sodium enters the cell. It is not improbable that death results from the disturbance in water and electrolyte metabolism.

An even greater similarity between the effects of the nitrogen mustards and radiant energy is obtained when actions on cell nuclei are studied. For example, cleavage of sea urchin eggs is arrested. If male Drosophila are exposed to low concentrations of nitrogen mustard, chromosomal abnormalities result in as high a percentage of sex-linked lethals as has been observed with *x*-ray and ultraviolet radiation. The effect of mitosis is the same as *x*-ray: arrest does not occur at a particular stage but the cell goes on to complete its mitosis, after which, however, no further mitotic activity occurs. The details of the chemical and biological actions of the nitrogen mustards have been recently summarized.[1]

Investigators have been impressed with the fact that it is the tissues which are undergoing the most rapid rate of cell division that are the most susceptible to the action of the mustards. Thus lymphoid tissue is particularly sensitive. It was only natural, therefore, that compounds which had such profound effects on lymphoid tissue should be evaluated in the therapy of lymphoma. Experimental work was cautiously started in 1942 at the New Haven Hospital. Preliminary reports of this and subsequent studies have appeared.[2,3] The results parallel those that can be obtained with *x*-ray. Perhaps the greatest success has attended the treatment of Hodgkin's disease. Remissions for varying periods up to eight months have been observed following single courses of treatment. Furthermore, favorable effects have been obtained in cases classified as roentgen ray resistant. There is some evidence that nitrogen mustard therapy may occasionally restore responsiveness to radiation. However, that resistance to the drug also develops is already evident.

In the treatment of lymphosarcoma the response is less predictable, but again similar to that observed with *x*-ray. Chronic lymphogenous leukemia appears to respond somewhat more favorably than chronic myelogenous leukemia but the results leave much to be desired. Little or no benefit is afforded in acute leukemia. In two reported cases of multiple myeloma, relief of bone pain was the only benefit noted.[3] Two patients with sympathoblastoma responded in a manner similar to *x*-ray.[3] Preliminary results in polycythemia rubra encourage further clinical trial.

With this brief analysis of the therapeutic results, I might say a few words as to the method of administration and the side reactions which occur. The nitrogen mustards are highly vesicant. They must be given intravenously and it is important that no extravasation occurs. They are most conveniently administered by injecting into the rubber tubing during the course of an intravenous drip of isotonic sodium chloride solution. The single dose is 0.1 mg. per kg.

body weight, but not to exceed 8 mg., given as a 0.1 per cent solution freshly prepared.

Local reactions at the site of injection are infrequent provided due precautions are taken, but occasionally thrombophlebitis develops. Nausea and vomiting are not uncommon, usually occurring two to three hours after injection and subsiding within the next few hours.

The first patients to receive nitrogen mustards were treated much too vigorously (ten daily doses) and exhibited severe granulocytopenia and thrombocytopenia. By reducing the total number of doses to three or four, given daily or every other day, it is possible to obtain an adequate therapeutic response without too severe a reaction on the bone marrow. However, a moderate degree of granulocytopenia and thrombocytopenia is always to be expected. A minor anemia may also develop.

The nitrogen mustards have introduced many more problems than they have solved. One of the most intriguing is the possible basic relationship between the actions of the nitrogen mustards and *x*-ray on the cell nucleus. They are so similar that one is led to believe that they must have some mechanism of action in common.

Another intriguing problem, especially from the pharmacological point of view, is the fact that the nitrogen mustards represent not one but rather a series of compounds of almost infinite number. Those that have been explored to date were developed as chemical warfare agents and therefore represent the most toxic members of the group. By changing substituent groups one can markedly affect distribution, reactivity, toxicity, and other important properties. Thus we have a means by which higher specificity of action within this series may be obtained.

The problem of combined therapy with nitrogen mustards, *x*-ray and radioactive isotopes still remains to be investigated. There have been a few instances where patients, resistant to radiation responded to nitrogen mustard. Subsequently their sensitivity to radiation returned. There have also been isolated cases where patients have been resistant to nitrogen mustard but sensitive to radiation. Thus there is the possibility that alternate therapy with nitrogen mustard and either *x*-ray or radioactive isotopes may solve the problem of resistance in the treatment of lymphoma. The principle of alternating therapeutic agents to prevent "fastness" in the chemotherapy of infectious disease is well established. A similar opportunity is now presented in the treatment of lymphoma.

There is another point of interest in this connection. The nitrogen mustards are so reactive that their effects are over within a matter of minutes. Thus whatever happens subsequent to their injection is the result of reactions which occur over a very brief period of time. This has been beautifully demonstrated by H. Smith and his co-workers who occluded the circulation to a given area for a period of five minutes after the injection of nitrogen mustard. Complete protection could be afforded to the bone marrow or the gastrointestinal tract by this technic. Therefore, the possibilities of combined treatment with radioactive compounds are intriguing in that with the nitrogen mustards one can get an initial effect which may be maintained with radioactive isotopes.

These are only a few of the problems which come to mind. In the clinical application of the nitrogen mustards we have gone only a short way in defining dosage and the courses of treatment that are best suited for a few syndromes. Biological investigators are little more advanced. At the present stage of their development, I would like to look upon the nitrogen mustards as a challenge to the clinician and biologist, the latter better to define their basic mechanism of action

and the former better to define therapeutic applications. To the pharmacologist is assigned the responsibility of forging a more potent chemotherapeutic weapon that may overcome the present limitations of this group of chemical warfare agents which has found therapeutic applications.

DR. TURNER: Are there any questions on this subject?

STUDENT: Has there been enough time to establish whether the cells tend to show less and less effect of treatment on protracted therapy? That occurs with *x*-ray.

DR. GILMAN: Yes, there has, and the response tends to become less marked with successive courses, but there again the possibility of alternating between radiant energy and the nitrogen mustards is opened up.

SAME STUDENT: Or alternating with different nitrogen mustard compounds?

DR. GILMAN: Or different compounds, possibly.

STUDENT: Has the effect of the nitrogen mustards on enzyme systems been studied?

DR. GILMAN: Yes, a variety of enzyme systems has been investigated. The most marked effects are exerted against the phosphokinases and indeed the British believe that this may represent the basic mechanisms of the vesicant action of this type of compound. However, it would be premature to attribute the actions of the nitrogen mustards on nuclear mechanisms to a specific enzymatic lesion.

SUMMARY

The term 'lymphoma' is applied to a variety of new growths having in common involvement of the spleen and lymph nodes but not otherwise known to be related. In Hodgkin's disease the lesion appears morphologically to resemble in many respects an infectious granuloma because of the variety of cell types involved; yet all efforts to demonstrate an etiological agent have so far failed. With the development of experimental animal technics in recent years, controlled investigations into this group of diseases may yield further information valuable to cancer research.

Radioactive isotopes and the nitrogen mustards have recently been introduced as agents of promise in the therapy of diseases of the lymphoma group. Isotopes of elements are atoms which have the same number of protons but differ in the number of neutrons in their nuclei, in accordance with their atomic weight. Hydrogen and heavy hydrogen are simple examples of this. The elements are distinguished by an ordered increase in the number of nuclear protons, in accordance with their atomic number. Hydrogen has only one proton, helium has two, lithium has three. As the atomic nucleus becomes more complex it also becomes more unstable. Natural substances with atomic numbers above 83 are so unstable that they undergo spontaneous nuclear disintegration by ejecting portions of their nuclei, a phenomenon called radioactivity. When atoms of substances below the atomic number of 83 are bombarded with nuclear particles from an outside source such as the cyclotron, a small number of normally stable atoms may take up extra particles in their nuclei. This produces an unstable nucleus which at some time breaks down with emission of alpha, beta or gamma radiation. This process is artificial radioactivity and such atoms are artificial radioactive isotopes.

Obviously, not all of the radioactive isotopes disintegrate in the same fashion or in the same length of time. But these factors are important ones which must be taken into account in order to calculate the amount of radiation given off and to estimate the dosage to be used. For the radioactive isotopes the unit of measure is the *millicurie*, or that amount of material which will insure the disintegration of 3.7×10^7 atoms per second. *X*-ray dosage, on the

other hand, is expressed in terms of *roentgens*. While these are different standards of measure, they may be correlated by the equation e.r. = 0.088 VT.

The nitrogen mustards developed during World War II are highly interesting compounds which seem to mimic the biologic effects of radiant energy. Lymphoid and granulocytic cells appear to be most sensitive to their action, then platelets, epithelial cells and other types in descending order of sensitivity. The remarkable similarity in results when cell nuclei are exposed either to irradiation or to the nitrogen mustards is striking. How either agent produces its effects is completely unknown.

The nitrogen mustards are highly vesicant. They must be surely placed within the vein, cause occasional thrombophlebitis nevertheless with nausea and vomiting, and in toxic doses, granulocytopenia, thrombocytopenia and anemia. When carefully administered they seem to produce about the same results in the lymphoma group as does irradiation. There is hope of increasing organ or system specificity by the proper use of substituent groups in the molecule. Also, they offer perhaps the most easily manageable form of therapy yet known in this group of disease. There is the further possibility that they may be combined with radiotherapy to achieve results beyond those so far obtained by either method alone.

Myeloid and lymphatic leukemia of the chronic types do well with either form of therapy. Acute leukemia, however, appears to be no more satisfactorily managed by the substances under discussion, than with the usual *x*-ray therapy. About the same may be said of Hodgkin's disease and the lymphosarcomas. Polycythemia on the other hand does well with the isotopes and gives indications of responding favorably to the nitrogen mustards.

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Clinico-pathological Conference

Acute Meningitis*

STENOGRAPHIC reports, slightly edited,† of weekly clinico-pathological conferences held in the Barnes Hospital are published in each issue of the Journal. These conferences are participated in jointly by members of the Departments of Internal Medicine and Pathology of the Washington University School of Medicine and by Junior and Senior medical students.

THE patient was a forty-three year old white married housewife who entered the Barnes Hospital for the first time on August 5, 1946. She was stuporous on admission and the history was obtained from her husband who stated that the patient's chief complaints were headache and fever. The family history was non-contributory. Except for smallpox many years previously, the patient had had no other illnesses or operations and had enjoyed excellent health. She had had no known recent exposure to an acute infectious disease.

Three weeks prior to admission, while vacationing in Michigan, she developed a fine red rash over the arms. No other symptoms were present at the time. Benadryl was prescribed by a physician who saw her and the rash disappeared within a few days. One week later the patient complained of headache and a severe pain between the shoulder blades. There was accompanying general malaise and her temperature was noted to be 101°F. Concomitantly a furuncle developed in the left upper lumbar region. The patient's symptoms persisted and seven days before entry to the Barnes Hospital, she was admitted to a hospital in a rural community in Illinois. A report from the physician who attended the patient there stated that on examination ptosis of the right eye and stiffness of the neck were observed.

Laboratory studies included normal white blood cell and differential counts. The urinalysis was normal. The patient was treated with penicillin and a sulfonamide but her temperature continued to rise to 103°F. daily. The furuncle enlarged in size but did not become fluctuant. The white blood cell count remained normal. Agglutination tests for typhoid fever, undulant fever, tularemia and typhus fever were negative. A roentgenogram of the chest showed nothing abnormal. On August 1, 1946, a lumbar puncture was performed. The spinal fluid was clear and contained five cells; a differential count was not reported. The patient failed progressively; ptosis of the right eye increased, stupor became deeper and the neck became stiffer. A second lumbar puncture was performed on August 5, 1946. Eighty-seven cells were present and all were lymphocytes. A Pandy test was positive; 100,000 units of penicillin were instilled intrathecally. A repeat chest roentgenogram showed the lung fields to be clear, but "the diaphragm was elevated suggesting a lesion beneath it." Because of the lack of response to therapy, the patient was transferred to the Barnes Hospital.

On entry her temperature was 39.6°C., pulse 110, respirations 24 and blood pres-

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sure 130/80. The patient was moderately obese, semi-stuporous, disoriented and resistant to examination. Her speech was uninterpretable. The skin was clear, except for a pigmented area the size of a silver dollar over the left upper flank. The head showed no evidence of trauma. There was ptosis of the right eye. The pupils were round; the left was slightly larger than the right but both reacted slightly to light. The patient could not look to the right as well as to the left. Examination of the fundi showed an area of old choroiditis on the right but no other abnormalities. There was thickening of the left ear drum. The pharynx could not be seen because the jaws were held rigid. The neck was rigid. Examination of the lungs revealed no abnormal findings. The heart was not enlarged and the rhythm was regular. A soft, Grade II, systolic murmur was heard over the precordium. The abdomen was soft; tenderness was noted in the epigastrium and left upper quadrant. Exquisite tenderness was elicited in the left costovertebral angle. The right arm was not used as well as the left. Abdominal reflexes, knee jerks and ankle jerks were not obtained. Bilateral Kernig signs were present. There were no pathological toe signs.

The laboratory data were as follows: Blood count: red cells, 5,770,000; hemoglobin, 16.1 Gm.; white cells, 15,500; differential count: eosinophiles, 1 per cent; stab forms, 13 per cent; segmented forms, 68 per cent; lymphocytes, 10 per cent; monocytes, 8 per cent. Urinalysis: albumin, trace; sediment, occasional granular cast. Kahn test: negative. Non-protein nitrogen: 26 mg. per cent. Blood culture: no growth.

Shortly after admission a lumbar puncture was performed. The initial pressure was 275 mm. of water; the final pressure, 125 mm. The fluid was xanthochromic. There were 270 cells without acid, many of which were crenated red blood cells; with acid there were 54 cells of which 22 were polymorphonuclear leukocytes and 34 were lymphocytes. No sugar was present in the fluid. The Wassermann test was negative and the colloidal gold curve was 1111111000. The fluid was sterile on culture. No pellicle was observed after two hours. Insufficient fluid was obtained for accurate protein determination but it ranged between 600 and 800 mg. per cent.

The patient was given intravenous fluids and closed urinary drainage was established. On the day after admission the neurological findings remained unchanged but tenderness was noted over the entire left abdomen. Because the same response was obtained from pinching the skin as on deep palpation, it was thought that the tenderness possibly represented superficial hyperesthesia. The patient's temperature remained high. The white blood cell count rose to 16,800, the differential showing 9 stab forms, 71 segmented forms, 10 lymphocytes and 10 monocytes.

On the third hospital day another lumbar puncture was performed. The initial pressure was 350 mm. of water; 14 cc. of clear yellow fluid was withdrawn and the final pressure was 150 mm. of water. Without acid 300 cells were present, of which a few were crenated red cells. With acid there were 270 cells, 50 per cent being polymorphonuclear forms and 50 per cent lymphocytes. The protein was 800 mg. per cent, sugar, 19 mg. per cent, and chlorides, 380 mg. per cent (626 mg. per cent as NaCl). The fluid was again sterile on culture. A pellicle developed in the spinal fluid; a hanging drop preparation showed no torula bodies. Smears of the pellicle were stained for acid fast bacilli but none were found.

The patient became more stuporous; her temperature rose to 40.8°C. Her respirations were shallow, forced and very rapid at 46 per minute. Examination of the lungs

revealed that breath sounds were diminished at both bases, more so on the left. Marked tenderness in the left upper abdomen persisted, and there was a suggestion of muscle guard. The patient's urinary output was fair. Reexamination of the eye grounds showed them to have remained unchanged. The patient's condition deteriorated rapidly, her arms and legs became cold, she became cyanotic, and she expired on August 8, 1946.

CLINICAL DISCUSSION

DR. HARRY L. ALEXANDER: This case is a very dramatic one. A woman, forty three years of age, whose general health had been excellent, became acutely ill, apparently with some type of meningitis, while enjoying a summer vacation. She died about two weeks later. The history records that three weeks before her death she developed a skin eruption for which she was given benadryl. In a few days, either because of the benadryl or spontaneously, the rash disappeared. It is true that benadryl does effect the urticarial type of lesion which is so common in the summertime. The first question to be asked is: Was there any relation between the eruption and her subsequent course? I feel that there was not for there was an interval of approximately one week after the disappearance of the rash and the onset of the more serious symptoms during which the patient felt well. If we conclude that the rash was incidental, it can be said that her illness began about July 24, 1946, two weeks before her admission to the Barnes Hospital. She complained of headache, fever, malaise and pain between the shoulders. She entered a hospital in rural Illinois where very thorough studies were made. The findings included a stiff neck, ptosis of the right eye, and a large furuncle in the left flank. Dr. Wood, do you believe that the neurologic findings and the furuncle were part of the same process, or do you think they were unrelated?

DR. W. BARRY WOOD, JR.: I believe that they were probably not related. It seems more likely that the meningeal reaction was on a basis other than that of staphylococcal infection which produced the furuncle. It is quite possible that the lesion in the central nervous system was an abscess which lay adjacent to the meninges and gave rise to a meningeal reaction. I do not believe, however, that the evidence indicates that this patient had staphylococcal meningitis.

DR. ALEXANDER: If this was an abscess, it apparently was not large because the fundi were normal even on the day before death. Would you comment on the spinal fluid findings, Dr. Moore, in regard to the possibility of a brain abscess? It is noted that in the hospital in Illinois, the spinal fluid obtained at the time of the second lumbar puncture revealed 87 cells of which all were lymphocytes. Would such a total and differential count be in keeping with the diagnosis?

DR. CARL V. MOORE: In the very early stage of an abscess perhaps, for at that stage the changes in the fluid may be variable and it is conceivable that lymphocytes would predominate. Usually, however, when there is irritation of the meninges because of an abscess, the percentage of polymorphonuclear leukocytes eventually rises.

DR. ALEXANDER: If we are to consider the furuncle of no importance in regard to the symptoms in the central nervous system, what, in your opinion, is the most probable diagnosis?

DR. C. V. MOORE: The very high spinal fluid protein is striking and on one occasion the spinal fluid sugar was zero. In meningismus, the spinal fluid is sterile and it is rare for the spinal sugar to be that low. If one were to rely on the experience accumulated by Merritt and Fremont-

Smith, he would arrive at the conclusion that this process was an infectious one which probably involved the spinal fluid even though organisms were not recovered.

DR. WOOD: According to work being carried on by Mr. Goldring, one of our senior students, there is experimental evidence that the presence of polymorphonuclear cells in the subarachnoid space will not bring the sugar down in the intact animal, although in vitro, the sugar may be lowered considerably by leukocytes. Apparently, in the intact animal, sugar enters the spinal fluid continuously and in the studies carried out to date, it has been impossible to lower the spinal sugar significantly by merely producing an inflammatory reaction in the subarachnoid space by the use of non-specific irritants.

It should be emphasized that this patient was given 100,000 units of penicillin intrathecally. Prior to instillation the spinal fluid cells were all lymphocytes. Such a dose of penicillin intrathecally is extremely large. 10,000 units is the dose usually recommended and 20,000 units is the upper limit. Some of the changes in the spinal fluid in this case may have been due to the large amount of penicillin introduced intrathecally.

DR. C. V. MOORE: Could the crenated red cells and the xanthochromic fluid be explained on that basis, Dr. Wood?

DR. WOOD: Possibly. There may have been sufficient injury to the capillaries in the subarachnoid space to allow passage of both protein and red blood cells into the cerebrospinal fluid.

DR. PAUL O. HAGEMAN: The xanthochromia may have been due to the color of the penicillin.

DR. ALEXANDER: Your suggestion as to the explanation of the xanthochromia fails to account for the fact that the red cells in the spinal fluid were crenated. Does xanthochromia occur in the spinal fluid in tuberculous meningitis?

DR. HAGEMAN: Yes.

DR. ALEXANDER: The spinal fluid chlorides were reported as 380 mg. per cent. Dr. Futcher, is that figure a little low?

DR. PALMER H. FUTCHER: 436–454 mg. per cent is the normal range for spinal fluid chlorides; they are thus slightly higher than the normal serum chlorides. In this case the spinal fluid chlorides were low.

DR. ALEXANDER: The combination of the spinal fluid findings here, low sugar, low chlorides, increased cell count with lymphocytes ranging from 50 to 100 per cent and a pellicle on standing is strongly suggestive of tuberculous meningitis.

DR. C. V. MOORE: I agree, but no organisms were found in the pellicle. Further the sugar of zero is not adequately explained, unless it fell as a result of the irritation of the penicillin.

DR. ALEXANDER: Do you consider the subsequent figure of 19 mg. per cent compatible with the diagnosis?

DR. C. V. MOORE: Yes.

DR. JOHN R. SMITH: Given one milliliter of spinal fluid containing many white cells, how long approximately will be required for the sugar to disappear?

MR. SIDNEY GOLDRING: In vitro, 10,000 cells per milliliter can reduce normal sugar values to zero within two hours. In this case the cell count was too low to be solely responsible for reducing the sugar.

DR. ALEXANDER: If this patient did not have tuberculous meningitis, what type of meningeal irritation or meningitis may give rise to a lymphocytosis in the spinal fluid and the neurologic findings recorded here.

DR. HAGEMAN: I believe that the clinical picture may have been due to staphylococcal sepsis; the findings are compatible with those which would result from an abscess adjacent to the meninges.

DR. WOOD: Where would such an abscess be located to give rise to the neurologic findings that this patient exhibited? These in-

cluded ptosis of the right eyelid, weakness of the right arm and sensory changes on the left, probably hyperesthesia.

DR. ALEXANDER: The hyperesthesia may have been a sequela of the furuncle for it was very extensive. Unfortunately a complete sensory examination was not recorded and it was merely noted that pressure over the left side of the abdomen caused a good deal of discomfort.

DR. WOOD: Dr. Hageman, are you considering a lesion in the left motor cortex?

DR. HAGEMAN: Yes.

DR. WOOD: The diffuse neurologic signs, particularly the ptosis, are difficult to explain on the basis of a cortical lesion. On the other hand if the third nerve was involved by meningitis, the ptosis could be explained. Third nerve lesions are commonly seen by the syphilologists; perhaps Dr. Scott can comment on the possible relationship of a cortical lesion to third nerve palsy.

DR. VIRGIL C. SCOTT: I do not believe that third nerve palsy can be explained by a cortical lesion.

DR. WOOD: Do you agree that a lesion in the meninges would explain the cranial nerve lesion?

DR. SCOTT: Yes.

DR. WOOD: I believe this point constitutes the strongest evidence in favor of the diagnosis of meningitis.

DR. ALEXANDER: Is third nerve involvement common in tuberculous meningitis?

DR. WOOD: Yes.

DR. FUTCHER: Dr. Alexander, is it not true that the white blood cell counts of only 7,600 and 8,000 recorded during the first part of the patient's illness, are against the diagnosis of staphylococcal infection. Certainly if there was staphylococcal bacteremia and a metastatic abscess in the brain, the white count should have been much higher.

DR. WOOD: That is a good point.

DR. ALEXANDER: Is tuberculous meningitis common in a patient in this age group?

DR. ROBERT A. MOORE: Tuberculous meningitis is certainly far more common in children than in adults.

DR. ALEXANDER: The course of the illness in this patient was two weeks; that seems very rapid for tuberculous meningitis.

DR. HAGEMAN: It is shorter than the usual course by at least fifty per cent.

DR. WOOD: Perhaps the rapid course could, in part, be accounted for by the additional meningeal irritation resulting from the large amount of penicillin which was introduced intrathecally.

DR. ALEXANDER: This patient had two chest roentgenograms which were said to have shown no evidence of tuberculosis. It is likely that she had a subcortical lesion which ruptured into the subarachnoid space.

DR. HENRY A. SCHROEDER: Although this course would be a rapid one for torulosis, the lesions in that disease may involve both the skin and the central nervous system and there may be similar spinal fluid findings.

DR. WOOD: What was the hospital diagnosis?

DR. ROBERT J. GLASER: The admission diagnosis was brain abscess but in view of the subsequent findings the final clinical diagnosis was tuberculous meningitis.

DR. WOOD: Bronchopneumonia would perhaps explain the leukocytosis which occurred terminally; it was not in keeping with tuberculous meningitis.

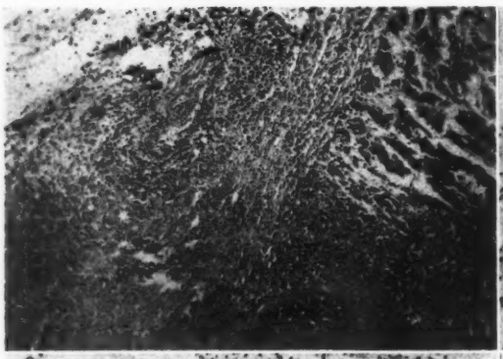
DR. ALEXANDER: It would appear that the consensus of opinion expressed in the discussion favors the diagnosis of tuberculous meningitis.

Final Clinical Diagnosis: Tuberculous meningitis.

PATHOLOGIC DISCUSSION

DR. RICHARD E. JOHNSON: At autopsy the body was that of a well nourished adult white woman. The significant findings were in the brain, the lung, and the adrenal

FIG. 1.



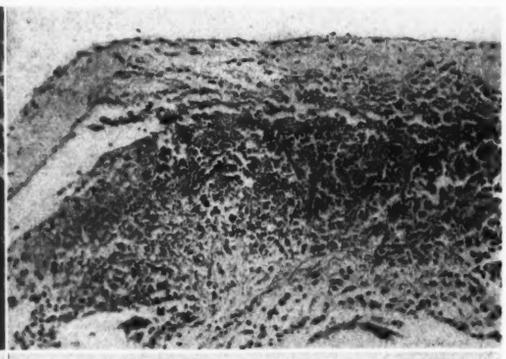
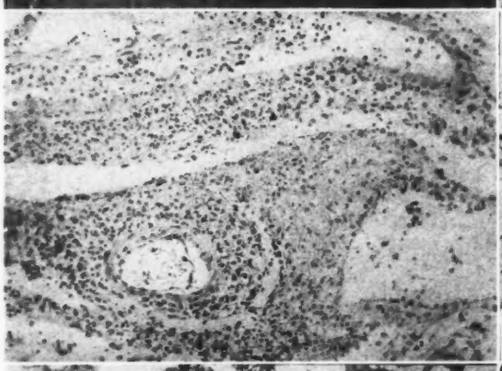


FIG. 2.

FIG. 3.



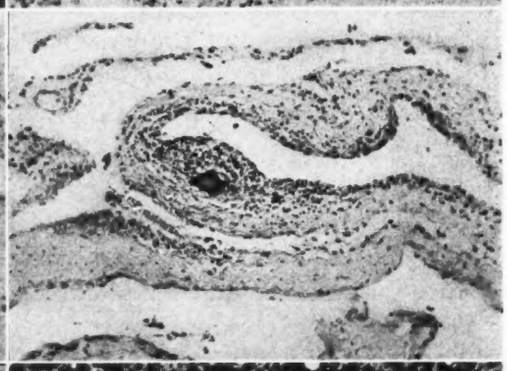
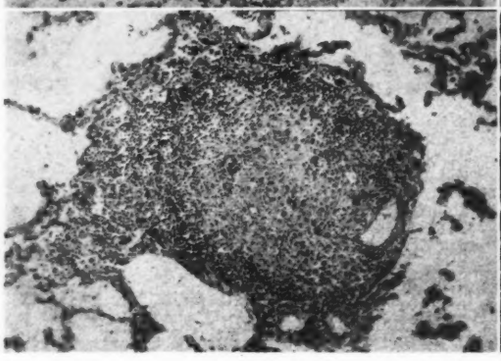


FIG. 4.

FIG. 5.



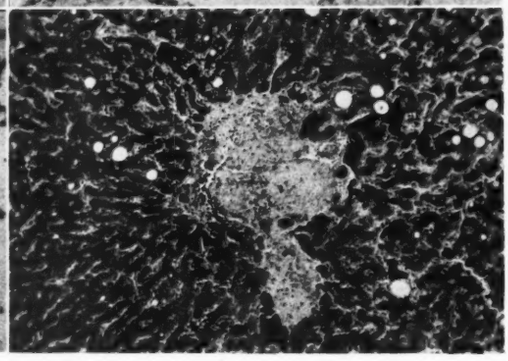


FIG. 6.

FIG. 1. Section of the right adrenal gland showing caseation necrosis extending through the cortex and involving periadrenal fat. × 100.

FIG. 2. Section of the meninges showing caseous necrosis of the exudate. × 100.

Fig. 3. Section showing characteristic changes of tuberculous meningitis involving a vessel in the meninges. × 100.

FIG. 4. Section through a meningeal vein in the wall of which a tubercle may be seen. × 100.

FIG. 5. Section of the lung showing early tubercle formation. × 470.

FIG. 6. Section of liver showing fibrous nodule, possibly a healed tubercle. × 470.

glands. The right adrenal weighed 22 Gm. and the left 5 Gm. The medullary portion of the right gland was completely replaced by yellowish-white, firm, cheesy material. In only one area, one cm. in diameter, it passed through the cortex, destroying the latter, and lay in apposition to the periadrenal fat. The medullary portion of the left adrenal gland was similarly involved.

The brain on gross examination showed slight opacity of the meninges over the base, extending from the optic chiasm back to the posterior edge of the pons. No tubercules were seen along the vessels in the Sylvian fissures or on the surface of the cerebellum. Multiple sections through the brain after fixation revealed no caseous foci similar to those seen in the adrenal glands. The left

lung was firmly bound to the parietal pleura by filmy fibrous adhesions which were easily broken. A fibrous pleural scar was noted in the apical portion. The right lung showed a few pleural adhesions. A calcified nodule was present in the periphery of the lower lobe and a cluster of calcified nodules in a bronchial lymph node. In the dependent portions of the lung, especially in the lower lobes, there were multiple, depressed, rubbery, purple foci which were interpreted as areas of atelectasis; in addition, diffusely throughout there were small, red, elevated foci, rather firm with a central yellow dot, interpreted as bronchopneumonia around the small bronchi.

DR. ROBERT A. MOORE: Dr. Johnson has presented to you our findings at the time of autopsy; in each adrenal gland there was a tuberculous process involving the medulla to a greater extent but extending out at one site into the cortex of the gland. In the meninges there was an inflammatory process which had led to increased opacity, but a diagnosis of tuberculous meningitis could not be made with any degree of certainty on the basis of the gross examination for the two most characteristic findings were lacking: the thick translucent type of exudate at the base of the brain, and the presence of numerous small tubercules throughout the meninges. In general, the diagnosis of tuberculous meningitis can be made if either one or both of these changes is observed.

In the microscopic study of the case, the first significant section (Fig. 1) is from the right adrenal gland where caseation necrosis is seen, extending through the cortex to involve the capsule and periadrenal fat. In the wall of the vein which lies outside the capsule, there is beginning necrosis. There is a fibrin thrombus occluding the lumen of the vessel. This finding is possibly of significance in explaining the pathogenesis of the meningitis, in view of Bateson's recent studies on the vertebral veins and their communications. In Figure 2 a section of the meninges is seen showing the arachnoid membrane, the subarachnoid space, and a portion of the brain; there is early caseation necrosis of the exudate in which most of the cells are mononuclear forms. Although the lesion is fairly characteristic the diagnosis of tuberculosis can not be made with certainty. In the next slide (Fig. 3) a more typical lesion of tuberculous meningitis is observed as evidenced by the formation of so-called tuberculous granulation tissue around the vessels in the meninges. The lesion is early for the epithelioid cells have a foamy type of cytoplasm and are not yet arranged radially about the vessel. Threads of fibrin are present in the subarachnoid space. The next section (Fig. 4) shows an excellent example of a tubercle in the wall of a meningeal vein. There is cellular infiltration and the tubercle projects directly into the lumen. There were several similar examples in other sections taken from the meninges.

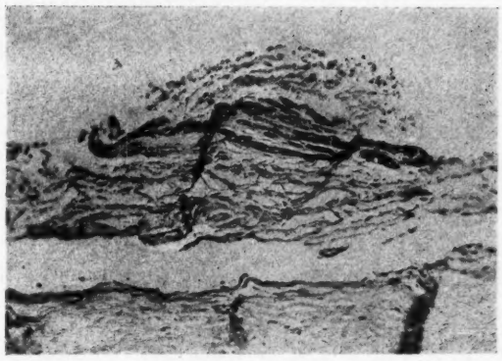


FIG. 7. Section from the right eye showing an area of chronic choroiditis.

A few early tubercles, composed of epithelioid cells, were scattered throughout the lungs. Giant cells had not yet formed, and the epithelioid cells were not yet organized into a well defined tubercle. (Fig. 5.) The gross diagnosis of bronchopneumonia was confirmed by microscopic study. A section of liver (Fig. 6) shows one of the lesions which were seen. There were nodules of fibrous tissue without epithelioid or giant

cells. They may be interpreted as healed tubercles.

Sections were stained for acid fast bacilli; organisms were seen in the adrenal gland and the meninges.

At the time of autopsy we were asked to remove a posterior segment of the right eye where a pigmented scar in the choroid was diagnosed as chronic or healed focal choroiditis. The microscopic section of that lesion (Fig. 7) confirmed the diagnosis.

The pathologic anatomy in this case is fairly evident. The calcified nodule in the periphery of the right lower lobe together with the calcified nodules in the tracheobronchial lymph nodes are generally accepted as evidence of primary tuberculous infection. The fibrous scar in the pleura of the apex of the left lung is not considered, in itself, to be of tuberculous origin unless associated with parenchymal involvement. In the adrenal glands there was active caseous tuberculosis with tubercle bacilli readily demonstrable. In the brain, tuberculous meningitis of an acute character, not fully developed, was present; it was consistent with a duration of two or three weeks. In the liver, there were a few tiny fibrous scars, interpreted as possible healed tubercles. The absence of similar lesions in the spleen, however, throws doubt on the interpretation.

With regard to pathogenesis, I do not know what the significance of the vertebral veins is in this situation. The accepted explanation for development of tuberculous meningitis is rupture, into the meninges or ventricular system, of a tuberculoma lying in the substance of the brain. Such a lesion could not be demonstrated in this case. In our experience, it has often been difficult to demonstrate a tuberculoma. The communication of meningeal and systemic venous channels through the elaborate network of vertebral veins described by Bateson offers an alternate pathway for extension of the tuberculous process.

Final Anatomic Diagnoses: Caseous tuberculosis of both adrenal glands; tuberculous meningitis; miliary tubercles in the lungs; bronchopneumonia, slight; focal atelectasis of the lungs; calcified nodules in lower lobe of right lung; calcified nodule in a right bronchial lymph node; fibrous pleural scar in apex of right lung; small pigmented nodule in fundus of right eye and brown pigmentation of skin in left lumbar region (history of furuncle seventeen days prior to death).

Case Report

Prostigmine Therapy in Hemiplegia*

JOSEPH C. DOANE, M.D., CHARLES H. KRAVITZ, M.D.

and LEONARD I. LAPINSOHN, M.D.

PHILADELPHIA, PENNSYLVANIA

THIS case report appears of interest because it seems to add weight to the suggestion that prostigmine represents an added therapeutic aid in the treatment of paralysis due to vascular accidents. In addition, it affords interesting speculative material relative to the theory that the nerve impulse is associated with, if not originated by the interaction of acetylcholine with other physiological components of the nervous mechanism.

CASE REPORT

On February 15, 1946, the patient, a white female, age thirty-nine, was admitted to the Jewish Hospital with the diagnosis of a recent cerebral vascular accident. A history of a rather marked hypertension covering a period of five years was obtained. About two and a half years prior to admission the patient awoke from an apparently sound sleep complaining of numbness in the left side of her face and weakness in her left arm. Following treatment for this condition she eventually regained full control of her limb. She was apparently well following this episode until her present illness, except for some varying degree of headache, nervousness, fatigue, and constipation, which her family physician attributed to hypertension.

On the night of admission the patient, becoming excited during a card game, complained of feeling generally ill and of numbness and tingling on the right side of her body. She became semi-stuporous and finally lapsed into complete unconsciousness, developing a flaccid paralysis of the right side of her body.

Physical examination revealed a well nourished female, with stertorous respiration, flushed face, confused and semi-stuporous. The heart sounds were increased in intensity, sinus tachycardia was present, and moderate left ventricular enlargement was elicited on palpation and percussion. There were no murmurs or thrills present. The eyes reacted to light but the left pupil was larger than the right and a conjugate deviation of the head and eyes to the left was noted. A central facial palsy was present. Flaccid paralysis of the right upper and lower extremity was present, with hyperactive deep and superficial reflexes in the upper, and absent reflexes in the lower extremity. There was slight nuchal rigidity and a suggestive Kernig sign. Spinal tap revealed a bloody spinal fluid with a pressure of 350 mm. of H_2O. The blood pressure on admission was 250/150, temperature 98.2°F., and the pulse and respiratory rates were 120 and 25 per minute, respectively.

Phlebotomy was performed on admission, with the removal of 400 cc. of blood. The blood pressure after phlebotomy was 180/140. Removal of 10 cc. of spinal fluid over a period of fifteen minutes caused a drop in spinal fluid pressure from 350 mm. to 90 mm. of H_2O. Following phlebotomy and spinal tap, the patient appeared to become more alert although still semi-stuporous. Dehydrating enemas and 50 per cent glucose, intravenously, were employed as indicated by signs of increased intracranial pressure. Further medication consisted of sodium nitrite, gr. iss, aminophylline, gr. iii, and phenobarbital, gr. ss four times a day. An electrocardiogram revealed no evidence of myocardial damage and eye ground examination revealed moderately advanced arteriosclerotic changes. The patient's course was a

* From Jewish Hospital, Phila., Pa.

stormy one from the time of admission to five days following her cerebral accident. She remained semi-stuporous, did not react well to stimuli, and her prognosis appeared to be extremely grave. On the sixth day following her admission, the patient became more alert, was able to recognize visitors and appeared to be improving somewhat. Her general condition improved greatly during the following week, but while the patient's prognosis as to survival steadily improved, there was no evidence of improvement in the movement of her paralyzed limbs, the physical signs remaining approximately the same as on admission. Nineteen days following admission there was still little or no improvement in the movement of her paralyzed limbs.

Barnes and Beutner[1,2] have suggested that cholinergic drugs should theoretically stimulate healing of central nervous system lesions. Ward and Kennard employed doryl in primates.[9] Jepson suggested the use of prostigmine in the treatment of infantile cerebral paralysis. It was decided to employ prostigmine in this case in an endeavor to hasten the restoration of limb function. Thirty mg. of prostigmine bromide with $\frac{1}{150}$ gr. of atropine sulfate were administered three times a day starting on March 5th, approximately nineteen days after admission. There appeared to be marked improvement in the patient's condition with increase of range of motion, which was manifested within twenty-four hours. However, nausea, abdominal cramps, and diarrhea supervened, whereupon atropine sulfate, gr. $\frac{1}{100}$, was administered which immediately relieved these symptoms. Prostigmine was discontinued for a day following this episode, and there seemed to be a regression in range of motion, strength and coordination of voluntary movement of the upper and lower limbs. The dose of prostigmine was then reduced to 15 mg. four times a day and $\frac{1}{2}$ gr. of phenobarbital was added to each dose, on the theoretical supposition that the latter drug would act as a depressant of any further untoward reactions.* This dosage appeared to cause no untoward reaction and the patient's condition rapidly improved. On March 10th she was able slowly to flex and extend her lower extremity and to raise her arm to an angle of 15 degrees, and lift it approximately two inches from the flat surface of her bed. Her seventh and twelfth nerve palsies were greatly improved. On March 11th the ability to flex and extend her leg partially increased in strength and speed of execution; she became able to touch her right finger to her nose, her grip became stronger and coordination improved greatly. On March 12th she was able to place her hand on the top of her head, and slowly flex, extend, abduct and adduct, pronate and supinate her right arm. Her speech and vision were also improved. On March 13th she was able completely to flex and extend her leg to an angle of 60 degrees against gravity in a sitting position, able to throw a ball, and her vision and speech were normal except for a slight nasal quality to her voice. On March 14th her condition was approximately the same. Her speech was somewhat less nasal in quality, and her right thumb and fingers appeared less spastic, and better coordinated. On March 16th she was able to turn from side to side in her bed. On March 17th she was allowed to sit in a chair by her bed for a half hour. On March 18th the patient attempted to stand, and on March 19th she was able to take a few steps. Her condition after this improved gradually until eight weeks after her discharge from the hospital on March 20th, she appeared to be clinically well except for slight weakness of her right arm, and a slight lack of coordination in her gait. Prostigmine was continued during her convalescence in the doses employed during hospitalization, and a close watch was kept on her progress during this period. Follow-up examination reveals that this patient has made a rather unusual recovery, in view of the severity of her cerebral accident and her poor prognosis on admission.

Of interest, also, in this case, is the sustained drop in blood pressure noted in conjunction with the administration of prostigmine and

* It has been shown experimentally by Barnes and Beutner[2] *in vitro* experiments that both phenobarbital and dilantin sodium give rise to positive currents in their oil cell experiments. This generation of a positive charge directly antagonizes the negative charge set up by acetylcholine, hence the rationale for the use of phenobarbital and dilantin in the treatment of epilepsy. The view that excess acetylcholine with resultant increased negativity and stimulation plays a part in the etiology of epilepsy has long been held by these investigators.

sodium nitrite. This lowering of arterial pressure has persisted during her convalescence rising but slightly on resumption of activity.

COMMENT

Kabat[6] has reported encouraging results using neostigmine in various cases of neuromuscular dysfunction. Of particular interest are his results in cases of hemiplegia of cerebral vascular origin, monoplegia, and cerebral palsy. This observer reports a decrease in spasticity, increased range of passive motion, decreased deformity, relief from muscle pain and increase in voluntary motion in his cases of hemiplegia. Improvement was noted in his cases of monoplegia and definite improvement of spasticity and some improvement in strength and coordination in the cases of cerebral palsy in which prostigmine was employed.

Kabat recommends the subcutaneous injection once or twice daily of 2 cc. of neostigmine methyl sulfate 1–2000 solution plus $\frac{1}{100}$ gr. to $\frac{1}{150}$ gr. of atropine sulfate.

One of us (J. C. D.) has recently successfully employed prostigmine bromide in oral doses of 15 to 30 mg. three times a day for the relief of spasticity in multiple sclerosis, and of postoperative hemiplegia in a patient with a pituitary gland tumor.

Jepson[5] treated twenty-five cases of infantile cerebral paralysis using oral prostigmine bromide in doses of 5 mg. three times a day. The medication was continued for at least six months or as long as improvement was noted. The chief results achieved in this series seemed to be a decrease in muscle spasm and an increase in function of the muscles involved.

The results noted in the references cited above are similar to the results noted in the case presented. The rapid rate of recovery seen in this patient coincided with the period during which prostigmine was administered.

Whether the drug brings about its therapeutic effect through relief of spasticity, as in treatment of arthritis and poliomyelitis, or whether actual aid in healing the central nervous system lesions is brought about, is not known. Ward and Kennard[9] present evidence that actual aid in healing results following the use of prostigmine. Doryl, a cholinergic drug, was used in their experiments to treat lesions of the central nervous system in monkeys, and here accelerated recovery of function was reported. Prostigmine 0.01 per cent has been used by Welsh[10] to aid in the regeneration of a cut planarian. He maintains that acetylcholine, a physiologic cholinergic agent, is a trophic substance maintaining the integrity of neurones and hence should aid in healing. Whether prostigmine acts by neutralizing choline esterase and thus allows excess acetylcholine to accumulate and achieve a therapeutic effect, or whether it has an independent cholinergic action of its own is as yet unknown. Evidence at hand seems to suggest that the latter supposition is correct.

Niker[8] et al. believe that prostigmine produces cholinergic effects of its own. After blocking choline esterase effects with a suitable physiological agent, they noted that muscular contractions could be elicited with prostigmine.

Eserine, a cholinergic drug which supposedly produces its effect solely by neutralization of choline esterase with subsequent increase in acetylcholine, has been shown to have less physiological effect than prostigmine both *in vitro* and *in vivo* experiments. Williams[11] reports that intravenous administration of prostigmine in much smaller amounts than eserine increased petit mal brain waves in epileptics. Barnes[2] found that the electrical potential set up by prostigmine in oil cell experiments is much more lasting than that caused by eserine. He states that the electrical effects of prostigmine are the factors important in nerve cell regeneration and that the action

currents thus set up aid in bridging neural connections.

Of note also in this case is the reduction in blood pressure coincident with the administration of the drug. Although prostigmine has been reported to have some peripheral vasoconstricting action by Mendez and Ravin,[7] still as a cholinergic drug the peripheral action should be primarily one of vasodilation. If the cholinergic action of prostigmine as a vasodilator is of use, as suggested by this case, in bringing about the lowering of blood pressure in selected cases of essential hypertension, then rich avenues of investigation are opened in view of the theories generally held that essential hypertension is of neurogenic origin.[4] Its main action, however, may be only the prevention of the overstimulation of sympathetic fibers, the antagonistic action of cholinergic and adrenergic drugs and their effects on peripheral vascular structures being an accepted physiological fact. This possible relationship of prostigmine therapy to the drop in blood pressure must as yet of necessity be limited to the sphere of theoretical speculation.

SUMMARY

A report of a patient with hemiplegia probably due to cerebral hemorrhage is presented. It seemed that excellent therapeutic results were obtained by using prostigmine bromide orally. Rapid return of strength, an increase in range of motion, and decreased spasticity, as well as a greatly improved general condition, were manifested promptly. The therapeutic results far surpassed the prognostic hopes held for the patient.

Reports of other patients thus treated and a brief discussion of theoretical implications involved are presented.

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Editorial

Rutin

One of the greatest difficulties in the evaluation of a new drug or biological product is securing proper control observations. This is especially so in the case of chronic disease which runs a variable and uncertain course such as hypertension or chronic hepatitis, or with disorders in which the complaints are mainly subjective, for example, migraine. What one needs, of course, are objective criteria of drug action, capable of measurement, and in the end, of statistical analysis.

The use of methionine in chronic hepatitis (cirrhosis) is a case in point. There is ample objective evidence that methionine has real protective and curative influence in certain types of experimental liver damage; in the human, on the other hand, it is difficult to secure convincing effects which have not been duplicated in other patients observed before the modern treatment of cirrhosis was introduced. Migraine illustrates the same problem. Here benefit or even cure has been claimed with every conceivable agent; and at the present time good results seem to be obtained by some with infusions of histamine, when at the same time other observers prefer to treat certain types of headache with so-called anti-histaminic drugs. The truth of the matter probably is that suggestion, chance, faith and intangible variables enter so much into the situation with these vague and ill defined conditions that really conclusive evaluation of therapeutic agents is almost impossible. It behooves the clinical investigator, therefore, to make every effort to select patients in whom the results of therapy can be gauged accurately.

Rutin, a new agent, for which certain therapeutic claims have been made with reference to disturbances of capillary fragility and permeability, presents similar problems. Rutin is said to be a glucoside of quercetin which can be obtained from various leafy plants and flowers. Buckwheat leaves have been the source of some of the available material. It is a flavone derivative related to those substances found in "citrin," an extract of lemon, which under the designation vitamin "P" has already been studied for its effects on capillary permeability, with dubious or certainly controversial results. The question has been raised as to whether rutin is the active substance in citrin. Just how rutin is supposed to act is not clear. When we recall that capillary permeability and so-called "fragility" may be altered either by disorders of the capillary membrane itself or on the other hand by changes in the cement substance between the endothelial cells, and when we realize that the cement substance in turn is affected by all sorts of variations in reaction and in concentration of various blood ions, it becomes clear how complicated the situation is and how difficult a really critical evaluation of any substance from the standpoint of its effect on "capillary fragility" must be. To call rutin vitamin "P" or give it such a general designation as the capillary permeability regulating vitamin hardly seems justifiable as yet.

Meanwhile, as pointed out recently,[1] reports have become prevalent that rutin is of value in the treatment of hypertension although it appears that no such claims

[1] Report of the Council on Pharmacy and Chemistry. Rutin. *J. A. M. A.*, 131: 743, 1946.

have actually been made in the published literature. It has been found, however, that the use of rutin may be followed by lessening of capillary permeability under certain conditions as measured by a standard petechiometer test.[2] Since the material seems to produce no toxic effects in man and is furnished in the form of small, practically tasteless pellets which can be taken simply by mouth, a thorough appraisal in clinical conditions in which capillary fragility is altered seems worth while. As far as hypertension is concerned, those seriously ill patients who have marked changes in the smaller vessels, especially in the eye grounds, with hemorrhage and edema certainly deserve the benefit of a trial with a harmless agent of this sort in the face of a prognosis which otherwise is absolutely bad. It is to be hoped, however, that those working with the material will select cases capable of evaluation and will make objective observations with meticulous care in order to learn as soon as possible what rutin really accomplishes.

There would also seem to be an interesting field in the laboratory for studies of the effect of rutin on experimental capillary damage in the light of modern knowledge of the pathological physiology of the minute vessels. It should be easy to devise experiments which would be decisive, especially if beneficial results were obtained.

A. L. B.

[2] SHARMO, R. L. Rutin: a new drug for the treatment of capillary fragility. *Am. J. M. Sc.*, 211: 339, 1946.

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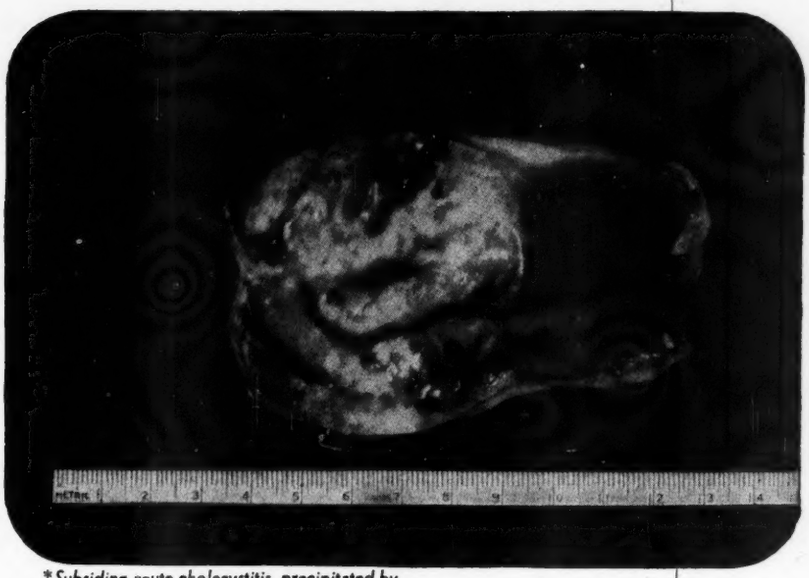

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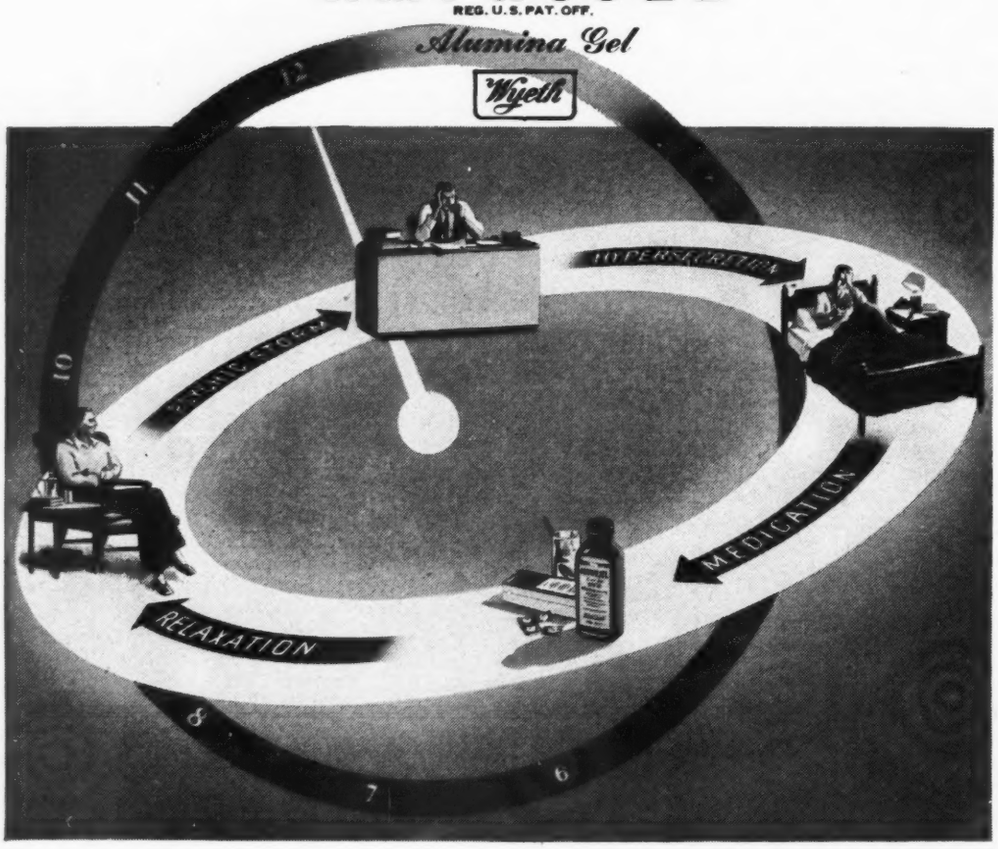

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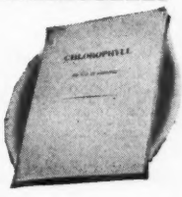

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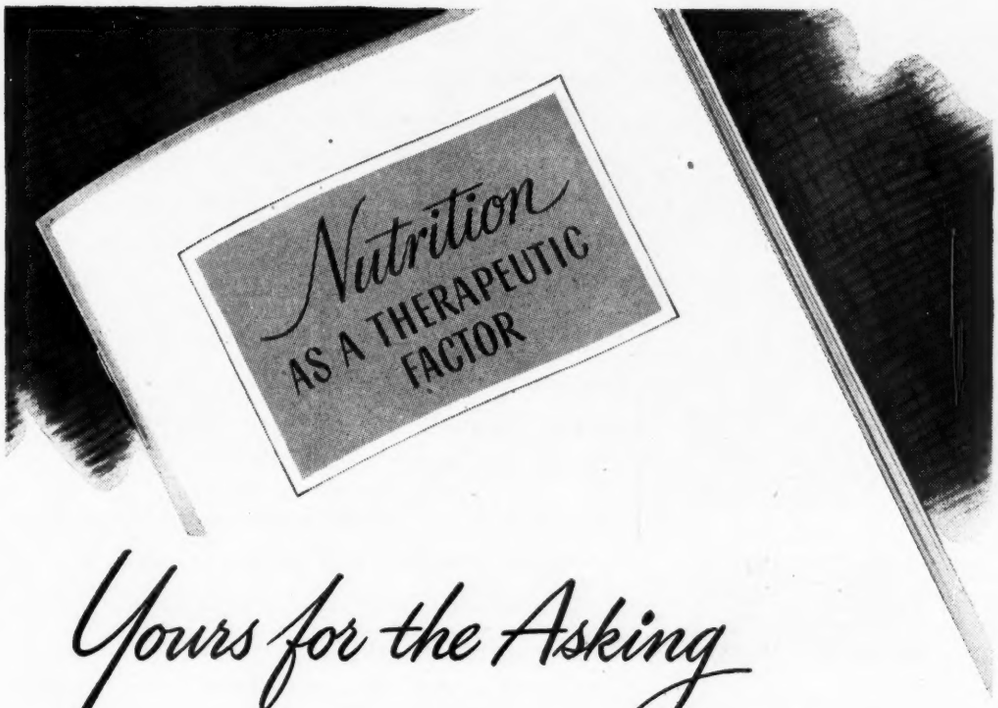

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